U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB-272 078

Effects of Cyanophage SAM-1 Upon 'Microcystis aeruginosa

Wisconsin Univ -Oshkosh

Prepared for

Corvallis Environmental Research Lab, Oreg Special Studies Branch

Jul 77

PB 272 078

EPA-600/3-77-070
July 1977

Ecological Research Series

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

RESEARCH REPORTING SERIES

Research reports of the Office of Research and Development, U.S. Environmental Protection Agency, have been grouped into nine series. These nine broad categories were established to facilitate further development and application of environmental technology. Elimination of traditional grouping was consciously planned to foster technology transfer and a maximum interface in related fields. The nine series are:

1. Environmental Health Effects Research
2. Environmental Protection Technology
3. Ecological Research
4. Environmental Monitoring
5. Socioeconomic Environmental Studies
6. Scientific and Technical Assessment Reports (STAR)
7. Interagency Energy-Environment Research and Development
8. "Special" Reports
9. Miscellaneous Reports

This report has been assigned to the ECOLOGICAL RESEARCH series. This series describes research on the effects of pollution on humans, plant and animal species, and materials. Problems are assessed for their long- and short-term influences. Investigations include formation, transport, and pathway studies to determine the fate of pollutants and their effects. This work provides the technical basis for setting standards to minimize undesirable changes in living organisms in the aquatic, terrestrial, and atmospheric environments.

This document is available to the public through the National Technical Information Service, Springfield, Virginia 22161.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1 REPORT NO. EPA-600/3-77-079	2
3 RECIPIENT'S ACCESSION NO. PB-271 078	
4 TITLE AND SUBTITLE: Effects of Cyanophage SAM-1 upon Microcystis aeruginosa	5 REPORT DATE: July 197
	6 PERFORMING ORGANIZATION CODE
7 AUTHOR(S): D. L. Parker, G. P. Jansen and L. Corbett	8 PERFORMING ORGANIZATION REPORT NO
9 PERFORMING ORGANIZATION NAME AND ADDRESS: University of Wisconsin - Oshkosh, Oshkosh, Wisconsin 54901	10 PROGRAM ELEMENT NO. 1BA031 11 CONTRACT/GRANT NO. P5J1190-J
12 SPONSORING AGENCY NAME AND ADDRESS: U.S. Environmental Protection Agency -- Corvallis, OR, Corvallis Environmental Research Laboratory, 200 S. W. 35th St., Corvallis, Oregon 97330	13 TYPE OF REPORT AND PERIOD COVERED: Final 14 SPONSORING AGENCY CODE: EPA/600/02

15 SUPPLEMENTARY NOTES

16 ABSTRACT

The isolation, propagation and application of Cyanophage SAM-1 to control blue-green algal blooms is discussed.

Cyanophage SAM-1, which infects Synechococcus cedrorum, Anacystis nidulans and certain strains of Microcystis aeruginosa has been isolated from sewage. The host range of cyanophage SAM-1 differs from those of other reported cyanophages. Phage SAM-1 stocks are rapidly inactivated at temperatures above 45°C or at pH values below 4 or above 10. The simultaneous application of chloroform and of agitation reduces viral infectivity to 20% of the original infectivity. The SAM-1 multiplication cycle has a latent period of 10 hours, a rise period of an additional 6 hours, and eclipse period of 6 hours, and an average burst size of 90 plaque-forming units per infected cell. Electron micrographs show SAM-1 virions consisting of polyhedral head and a contractile tail with a distinctive terminal structure. The properties of cyanophage SAM-1 are compared with those of other cyanophages.

17 KEY WORDS AND DOCUMENT ANALYSIS

a DESCRIPTORS	b IDENTIFIERS/OPEN ENDED TERMS	c COSATI Field/Group
cyanophage viruses blue-green algae eutrophication isogeny		02I 04A

18 DISTRIBUTION STATEMENT: RELEASE UNLIMITED	19 SECURITY CLASS (This Report): UNCLASSIFIED 20 SECURITY CLASS (This page): UNCLASSIFIED	21 NO. OF PAGES: 69 22 PRICE: PC A04 - A01

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

EPA-600/3-77-079
July 1977

EFFECTS OF CYANOPHAGE SAM-1 UPON *MICROCYSTIS AERUGINOSA*

by

D. L. Parker, G. F. Jansen, and L. Corbett
University of Wisconsin - Oshkosh
Oshkosh, Wisconsin 54901

Project PS.11190-J

Project Officer
William E. Miller
Special Studies Branch
Corvallis Environmental Laboratory

CORVALLIS ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CORVALLIS, OREGON 97330

DISCLAIMER

This report has been reviewed by the Special Studies Branch, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

FOREWORD

Effective regulatory and enforcement actions by the Environmental Protection Agency would be virtually impossible without sound scientific data on pollutants and their impact on environmental stability and human health. Responsibility for building this data base has been assigned to EPA's Office of Research and Development and its 15 major field installations, one of which is the Corvallis Environmental Research Laboratory (CERL).

The primary mission of the Corvallis Laboratory is research on the effects of environmental pollutants on terrestrial, freshwater, and marine ecosystems; the behavior, effects and control of pollutants in lake systems; and the development of predictive models on the movement of pollutants in the biosphere.

This report centers around the isolation, culture and use of viruses as regulators of blue-green algal blooms in natural waters.

A. F. Bartsch
Director, CERL

ABSTRACT

Cyanophage SAM-1, which infects Synechococcus cedrorum, Anacystis nidulans and certain strains of Microcystis aeruginosa has been isolated from sewage. The host range of cyanophage SAM-1 differs from those of other reported cyanophages. Phage SAM-1 stocks are rapidly inactivated at temperatures above 45°C or at pH values below 4 or above 10. The simultaneous application of chloroform and of agitation reduces viral infectivity to 20% of the original infectivity. The SAM-1 multiplication cycle has a latent period of 10 hours, a rise period of an additional 6 hours, and eclipse period of 6 hours, and an average burst size of 90 plaque-forming units per infected cell. Electron micrographs show SAM-1 virions consisting of polyhedral head and a contractile tail with a distinctive terminal structure. The properties of cyanophage SAM-1 are compared with those of other cyanophages.

Four distinct morphological types of M. aeruginosa have been isolated from the Winnebago Pool, Wisconsin. These types "breed true" upon cultivation and can be enumerated in water samples, by counting of characteristic colonies growing on Jansen's agar. The virus-sensitivities, frequencies on different dates, frequencies at different stations and other properties of these M. aeruginosa types are discussed.

The potential role which cyanophage SAM-1 may play in the limitation or in the production of Microcystis blooms is discussed in terms of virus-sensitivities and their relationship to possible lysogeny.

This report was sumbitted in fulfillment of purchase order #P5J1190J by Dr. Parker under the sponsorship of the U.S. Environmental Protection Agency. This report was completed as of July 15, 1976.

CONTENTS

	Page
FOREWORD	iii
ABSTRACT	iv
LIST OF TABLES	vi
LIST OF FIGURES	vii
1. INTRODUCTION	1
2. MATERIALS AND METHODS	6
Maintenance of Algal Cultures	
Measurement of Light Intensity	
Standard Growth Conditions	
Standard Plaque Assay	
Spot Test for virus-sensitivity of Blue Green Algae	
Isolation and Propagation of Cyanophage SAM-1	
Unialgal cultures of M. aeruginosa (morphological types)	
3. RESULTS	19
Host range of cyanophage SAM-1	
Plaque morphologies and rates of plaque appearance	
Stability of cyanophage SAM-1	
SAM-1 Multiplication Cycle: One-step growth experiment	
SAM-1 Multiplication Cycle: Premature lysis experiment	
Electronmicrographs of cyanophage SAM-1	
Properties of M. aeruginosa cultures from the Winnebago pool	
Virus-sensitivities of M. aeruginosa morphological types	
Frequency of M. aeruginosa morphological types at different stations and times	
Unexplained lytic phenomena	
4. DISCUSSION	45
Properties of cyanophage SAM-1	
Topics for further experimentation	
5. REFERENCES	49

LIST OF TABLES

Table		Page
1	Properties of certain cyanophages	8
2	Comparison of cyanophages of Chroococcales	3
3	Algal cultures	9
4	Jansen's medium	12
5	Host ranges of several cyanophages	20
6	SAM-1 plaques on susceptible hosts	21
7	Stability of cyanophage SAM-1 in water, $MgCl_2$ and NaCl	22
8	Comparison of M. aeruginosa morphological types	35

LIST OF FIGURES

Figure		Page
1	The Winnebago Pool	7
2	Locations of stations	8
3	Stability of phage SAM-1 at different temperatures	25
4	Stability of phage SAM-1 at different pH values	26
5	Sensitivity of phage SAM-1 to chloroform and to agitation	26
6	SAM-1 multiplication cycle	27
7	Electronmicrograph of SAM-1 virion	29
8	Electronmicrograph of SAM-1 virion	30
9	Electronimicrograph of SAM-1 virion with contracted tail	30
10	Colony of M. aeruginosa C1, on Jansen's agar	31
11	Colony of M. aeruginosa C2, on Jansen's agar	31
12	Colony of M. aeruginosa C3, on Jansen's agar	32
13	Colony of M. aeruginosa C4, on Jansen's agar	32
14	Culture of M. aeruginosa C1, in liquid medium	33
15	Culture of M. aeruginosa C2, in liquid medium	33
16	Culture of M. aeruginosa C3, in liquid medium	34
17	Culture of M. aeruginosa C4, in liquid medium	34
18	Frequency of M. aeruginosa cells at different stations	37
19	Frequency of M. aeruginosa types, station 1	38
20	Frequency of M. aeruginosa types, station 2	39
21	Frequency of M. aeruginosa types, station 3	40

LIST OF FIGURES (Con't.)

Figure		Page
22	Dissolved oxygen at each station	41
23	Water temperature at each station	42
24	Secchi disc readings at each station	43
25	Unidentified lytic agent affecting M. aeruginosa J1	44
26	Unidentified lytic agent affecting Synechococcus sp.	44

SECTION 1

INTRODUCTION

The susceptibility of the Cyanophyta, or blue-green algae, to viral infection was first established by Safferman and Morris (1963), when they reported the isolation of cyanophage LPP-1. Since that time, the study of cyanophages has expanded sufficiently to be the topic of several recent review articles (Brown, 1972; Safferman, 1973; Padan and Shilo, 1973).

Tables 1 and 2 summarize the properties of certain cyanophages which have been extensively characterized. These viruses can be grouped on the basis of host range, serologic cross reactions and structure of the virion, also called the extracellular virus particle. The virions of all cyanophages thus far examined contain linear, double-stranded DNA and show a "head-plus-tail" structure (Brown, 1972; Padan and Shilo, 1973). Three general types of virion tails have been observed: (a) the short, non-contractile, but perhaps extendable, tails or collars of viruses LPP-1, LPP-2 and SM-1, (b) the longer contractile tails of viruses N-1 and AS-1, and (c) the long, non-contractile tail of virus S-1 (Brown, 1972; Safferman, 1973; Padan and Shilo, 1973).

The several cyanophage groups each have rather narrow host ranges (Table 1). Taking into account the existence of resistant variant cells and of host range mutant viruses, this specificity of the cyanophages could aid in identification and in typing of the Cyanophyta. Thus, the cyanophage might be a useful tool in reorganization of cyanophyte systematics, in the same way that Staphylococcus phages have been used for typing strains of S. aureus and other related species (Williams and Rippon, 1953).

Ecological studies indicate that cyanophages have a wide geographic distribution (Safferman and Morris, 1964b; Padan and Shilo, 1969; Shane, 1971; Shilo, 1971, 1972; Singh, 1973). Of twelve waste stabilization ponds sampled, Safferman and Morris (1967) detected LPP viruses in eleven ponds. Similar viruses have been isolated from widely distributed sites in the United States (Jackson, 1967; Safferman, 1968; Jackson and Sladecek, 1970; Shane, 1971; Cannon, et al., 1974), from fish ponds in Israel (Padan and Shilo, 1969), from stabilization ponds and streams in Scotland (Daft et al., 1970) and from water bodies in India (Singh and Singh, 1967). Viruses of the SM-1, MA-1 group have been isolated both in the United States (Safferman et al., 1969) and in Russia (Goryushin and Chaplinskaya, 1966, 1968). Cyanophage AS-1 has been recovered at least twice, once from Florida by Safferman et al., (1972) and again from Missouri by Sherman (1975). Cyanophages are characteristically more prevalent in polluted or eutrophic waters than in less polluted or hypotrophic waters (Safferman and Morris, 1964b, 1967; Singh and Singh, 1967; Padan and Shilo, 1969; Singh, 1971; Safferman et al., 1972; Shane et al., 1972; Shilo, 1972; Singh, 1973, 1974; Cannon et al., 1974).

TABLE 1 [illegible]

Class	Reference	Source	Name / Notes
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]

TABLE 2 [illegible]

	5-1	3A-1	8S-1	3M-1
[illegible]				
[illegible]	-	[illegible]	[illegible]	[illegible]
Edge-edge distance	32 Å	67 Å	32 Å	
Tail	non-[illegible]	short, if present	[illegible] sheet right core base plate	[illegible] sheet right core [illegible] structure
Length, width	140 Å	-	262.5 Å, 22.5 Å	-
Tail length/Rod diameter	2.8	-	2.7	2.5
Tail length/tail width	-	-	10.8	9
STABILITY OF [illegible]				
In distilled water	-	stable	[illegible] stable	stable
Ni^{2+} required	-	no	no	no
pH range of protein stability	-	pH 5–7	pH 4–10	pH 4–10
Temperature range of protein stability	-	4–45C	-	4–45C
Inactivation		55 C	60 C	55 C
REFERENCE	Hulpp et al. Nature [1972]	Sofroniew et al. [1981]	Sofroniew et al. [1981]	

Several cyanophages have been isolated from water samples taken during algal blooms or fish kills (Goryushin and Chaplinskaya, 1966, 1968; Singh, 1967; Padan and Shilo, 1969, 1973).

The widespread occurrence of cyanophages suggests that they may significantly influence the cyclic succession of cyanophyte populations in nature. Viruses might affect cyanophyte populations in several ways. First, free virus particles (virions) persisting in a body of water might infect and lyse susceptible host cells, thus limiting the host density (Safferman and Morris, 1964b, 1967; Goryushin and Chaplinskaya, 1966, 1968; Padan and Shilo, 1969; Jackson and Sladecek, 1970). Second, several cyanophages can enter "lysogeny," in which a non-infectious viral entity persists inside infected cells without producing any major effect on cell growth, division or survival (Singh et al., 1969, 1972; Cannon et al., 1971, 1972; Padan et al., 1972; Cannon, 1975; Rimon et al., 1975). Certain environmental conditions can simultaneously "induce" virus multiplication and subsequent cell lysis within most cells of a lysogenic population, with the result that a dense and apparently healthy culture can suddenly lyse (Cannon, 1975; Rimon et al., 1975). Such induction of virus multiplication in lysogenic cultures could play a role in the rapid decline of algal blooms, or it could be involved in the production of certain apparent blooms, characterized by moribund cells floating on the water surface. In the latter case, lysis of a dense but dispersed population could cause the cells to float upward and become concentrated at the surface. Evidence concerning the potential roles of virions and of lysogenic cells in cyanophyte population dynamics is considered in the following two paragraphs.

That an equilibrium lytic infection involving persisting virions may limit cyanophyte populations has been proposed by Safferman and Morris (1967) and by Padan and Shilo (1969), both of whom observed that susceptible algal species were never dominant in ponds containing significant concentrations of virions infecting those species. Safferman and Morris (1964) demonstrated that cyanophage LPP-1 could limit Plectonema populations under conditions stimulating natural algal blooms. A similar phenomenon was reported by Jackson and Sladecek (1970), who suggested that naturally-occurring virions in 5000-gallon (18,900 L) sewage treatment tanks were responsible for their repeated inability to produce Plectonema blooms in these tanks. Goryushin and Chaplinskaya (1966, 1968) observed that certain areas of the Kremenchug reservoir had clear areas even during the height of a Microcystis bloom and that these areas contained a virus infecting Microcystis. Furthermore, Russian investigators have reported success in using this virus to clear Microcystis blooms in a large reservoir (Padan and Shilo, 1973).

Lysogeny by cyanophages LPP-1D, LPP-2SP1, A5-1 and N-1 has been reported (Singh et al., 1969, 1972; Cannon et al., 1971, 1972; Padan et al., 1972; Cannon, 1975; Rimon et al., 1975). The probability that infected cells will enter the lysogenic cycle rather than the lytic viral multiplication cycle is frequently increased by environmental stress factors such as antibiotics, $HgCl_2$, $CuSO_4$, and nitrogen starvation (Cannon et al., 1972; Cannon, 1975). Lysogenic cells carrying a temperature sensitive mutant of LPP-2SP1 can be induced to produce virus by increasing the growth temperature (Rimon et al., 1975). Lysogenic cells carrying LPP-1D, A5-1 or N-1 can be induced to

release virus by the addition of mitomycin C (Cannon et al., 1971, 1972; Cannon, 1975). Lysogeny may play a significant role in the persistence of cyanophages in certain habitats. It might explain the rapid decline or rapid formation of certain algal blooms. Lysogeny might also influence determinations of viral host ranges, because lysogenic cells can not ordinarily be lysed by a virus which they are carrying, and thus would incorrectly be considered not to be a host for that virus.

In the present communication, a new cyanophage infecting Lyngbya aerugineum, Anacystis nidulans and Microcystis aeruginosa is described. In accordance with the established system of nomenclature (Safferman and Morris, 1963), this virus has been designated cyanophage SAM-1. Because of the unusual observation that only certain M. aeruginosa isolates are sensitive to SAM-1 virus, several additional M. aeruginosa strains have been isolated, classified into four morphological types, and tested for virus-sensitivity. The potential role which cyanophage SAM-1 may play in the limitation or in the production of Microcystis blooms is discussed in terms of these virus-sensitivities and in relation to possible lysogeny.

SECTION 2

MATERIALS AND METHODS

Lake Winnebago, which borders the eastern side of Oshkosh, Wisconsin, U.S.A., was the major source of the Cyanophyta. Lake Winnebago is the largest body of water within the boundaries of the state and is one of the largest freshwater lakes in the United States. Birge and Juday (1914) termed Lake Winnebago a glacial dam lake having an area of 208.3 mi^2 (557.7 km^2), and a maximum depth of 6.4 m. The lake has a continuous mixing pattern thoughout the ice-free months of the year. Lake Winnebago is part of the "Winnebago Pool," a complex, shallow reservoir having an area of 265 mi^2 (708 km^2) and comprising Lake Butte des Morts, Winneconne and Poygan (Figure 1). The pool is in the watershed of the Fox and Wolf Rivers, which join in Lake Butte des Morts and which exit Lake Winnebago through the lower Fox River to Green Bay and Lake Michigan (Sloey, 1970).

The Fox-Wolf watershed comprises some 6,000 mi^2 (15,530 km^2) and is the largest basin to Lake Michigan (Sloey, 1970). The Winnebago Pool retains some 20×10^9 ft^3 (70×10^6 m^3) of water (Olcott, 1966) and is presently the source of domestic water for the cities of Oshkosh, Neenah-Menasha and Appleton, which have a combined population of 150,000 (Sloey, 1970).

Three stations were sampled for both viral and planktonic algal content during this study (Figure 2). Stations one and two are located in the Fox River; upstream, station 1, and downstream, station 2, from the city of Oshkosh's sewage treatment plant waste discharge. Station three is located in Lake Winnebago near Oshkosh's water departments pretreatment basin.

The Fox-Wolf River System is the greatest contributor of nutrients to Lake Winnebago (EPA, 1974). In addition, there are numerous smaller tributaries which further contribute to the input of nutrients. These smaller tributaries flow through various types of ecological habitats bordering the lake, notably urban, agricultural and marsh areas. The Winnebago Pool is hypereutrophic (Sloey, 1970); and supports growth of numerous cyanophytes, each of which exhibits a particular seasonal fluctuation (Sloey and Blum, 1972).

MAINTENANCE OF ALGAL CULTURES

Table 3 lists each algal culture, its source and its reference number in the University of Wisconsin-Oshkosh culture collection. Isolation of M. aeruginosa morphological types C1, C2, C3 and C4 is considered under the appropriate heading below.

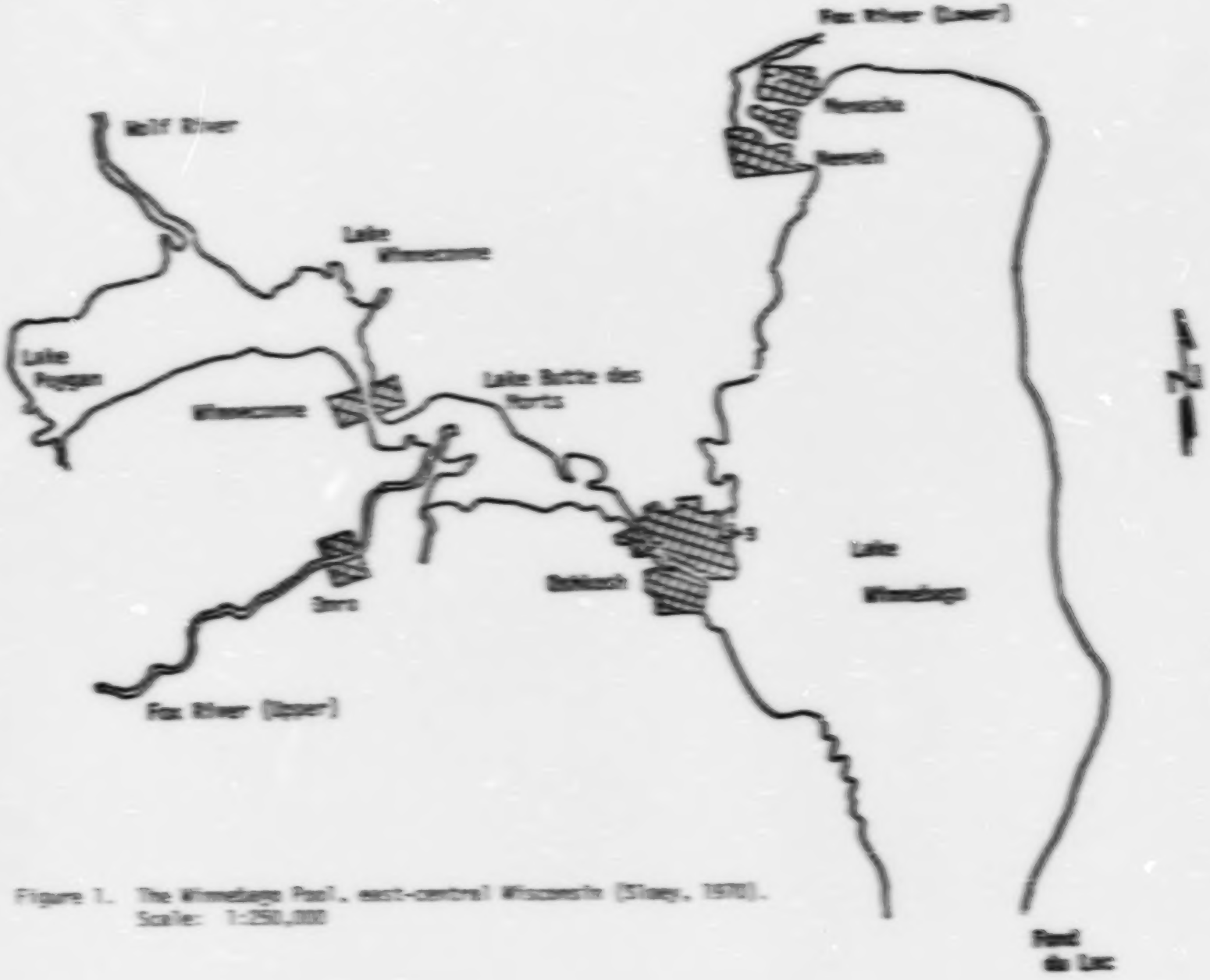

Figure 1. The Winnebago Pool, east-central Wisconsin (Stoertz, 1978).
Scale: 1:250,000

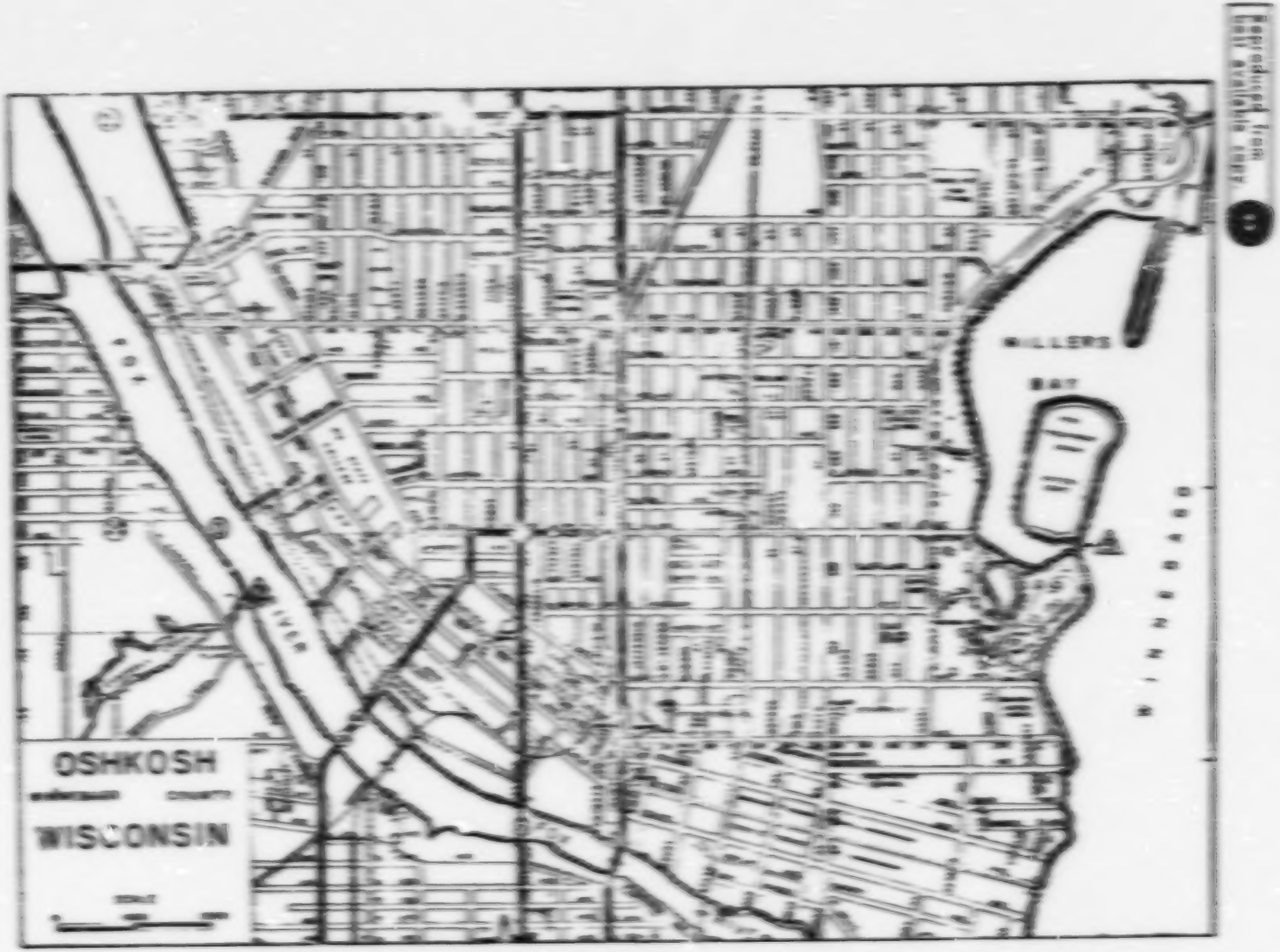

Figure 2. Locations of stations 1, 2, and 3. Symbols: ● Station 1, ■ Station 2, ▲ Station 3.

TABLE 1 ALGAL CULTURES

UNIALGAL CULTURES (NOT AXENIC)			AXENIC CULTURES DERIVED FROM THAT CULTURE	
Name as arrived at UW-M	UW-M (original)	Source	Axenic culture derived by	UW-M Axenic
Synechococcus elongatus UTEX #563	S2	R. Sieffermann E.P.A. Cincinnati 7/15/74	L. Corbett	S2CC
Synechococcus radiorae UTEX #1037	S3	R. Sieffermann E.P.A. Cincinnati 7/15/74	G. P. Jensen J. McGarin L. Corbett	S3GJ S3MG S3CC
Synechococcus cedrorum J3	J3	Lake Kinneret G. P. Jensen 4/15/73	G. P. Jensen L. Corbett	J3GJ J3CC
Anacystis nidulans UTEX #625	AC1	R. Sieffermann E.P.A. Cincinnati 7/15/74	L. Corbett	AC1CC
Anacystis sp. [Probably nidulans] JA	JA	Lake Kinneret G. P. Jensen 3/20/73	G. P. Jensen	JAGJ
Gloeocapsa calcarea Tilden 1898	G1	Lake Kinneret G. P. Jensen 8/15/74	-	-
Merismopedia elegans	Me1	Lake Kinneret G. P. Jensen 8/15/74	-	-
Oscillatoria subbrevis Schmidle 1901b	Os1	Lake Kinneret G. P. Jensen 8/15/74	J. McGarin	Os1MG
Anabaena flos-aquae [Lyngb.] de Brébisson	Ab1	Lake Kinneret G. P. Jensen 6/10/73	G. P. Jensen J. McGarin	Ab1MG

[illegible]

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

[illegible]

Table 4 gives the composition of Jansen's medium. Jansen's liquid medium is prepared by mixing 2 parts of sterile solution A, 5 parts of sterile solution B and 3 parts of sterile glass-distilled water. Jansen's agar is prepared by mixing 2 parts of sterile solution A, 5 parts of sterile solution B and 3 parts of sterile, melted 3.33% w/v Bacto agar (Difco Co). Solutions A and B are autoclaved separately and stored in opaque containers.

Jansen's cycloheximide agar is Jansen's agar containing 30 ug of cycloheximide (Sigma Co.) per ml. This concentration of cycloheximide inhibits the growth of eukaryotes but not of prokaryotes.

Tris hydroxymethylamino ethane (Tris) was dissolved to 0.01 M in glass distilled water, adjusted to pH 8.2 and autoclaved.

Measurement of light intensity

A Panlux light meter (Gossen-Ascor Co.) was used. The light intensity for liquid cultures was determined at the culture's mintcus, at the outside surface of the glass container. The light intensity for petri plate cultures was determined at the agar surface on which the colonies were growing, by placing the light meter inside the plastic bag and either 1 mm below the agar surface (for inverted plates) or at the level of the agar surface (for non-inverted plates).

Standard growth conditions

All cultures were propagated at 26-27°C and were illuminated from above by Westinghouse CW 40 fluorescent lamps. Petri plates were incubated in plastic bags, which were folded once at their openings and which contained moist paper towels below the petri plates. Unless otherwise specified, cultures were not agitated. Erlenmeyer flasks, vials and test tubes containing liquid cultures were filled to 1/5 of their maximum capacity and were loosely capped. Vials and test tubes are incubated on their sides, at 20° from the horizontal. Sterile media, sterile glassware and aseptic technique were used throughout.

Standard plaque assay

Ten-fold serial dilutions of the virus suspension were prepared in Jansen's liquid medium. One tenth ml of each viral dilution was added to 4 ml of a 3-day-old culture containing 1 x 10^8 cells/ml of the host organism. After adsorption for 1 hour at 26°C, the virus-host mixture was combined with 6 ml of melted (40°) Jansen's agar, then poured into a 15 x 100 mm petri plate already containing 25 ml of solidified Jansen's agar. The plates were incubated top-side-up overnight at 120 ft-c and at standard growth conditions, then inverted and incubated as before. The number of plaque forming units/ml (pfu/ml) was calculated from the number of plaques and the dilution factor, with S. cedrorum J3G1 as the host. The pfu/ml for phages AS-1 and SM-1 were similarly calculated, except that the hosts were S. cedrorum S1G1 and S. elongatus S2C2 respectively.

TABLE 4 JANSEN'S MEDIUM

SOLUTION A	
$NaNO_3$	670 mg
$Na_2HPO_4 \cdot 12H_2O$	17 mg
$K_2HPO_4 \cdot 3\ H_2O$	50 mg
$Na_2SiO_3 \cdot 5\ H_2O$	75 mg
$MgSO_4 \cdot 7\ H_2O$	100 mg
$MgCl_2 \cdot 6\ H_2O$	50 mg
Tricine buffer (Sigma Chem. Co.)	250 mg
2x distilled water	200 ml
1 N NaOH	to pH 8.3
SOLUTION B	
$FeCl_3 \cdot 6\ H_2O$	5.00 mg
$CaCl_2 \cdot 2\ H_2O$	42.00 mg
Na_2 ethylenediaminetetraacetate	4.00 mg
H_3BO_3	3.20 mg
$MnSO_4 \cdot 4\ H_2O$	3.40 mg
$ZnSO_4 \cdot 7\ H_2O$	0.30 mg
$(NH_4)_6\ Mo_7\ O_{24} \cdot 4H_2O$	0.08 mg
$CuSO_4 \cdot 5\ H_2O$	0.13 mg
$Co(NO_3)_2 \cdot 6\ H_2O$	0.15 mg
$Al_2\ K(SO_4)_4 \cdot 24\ H_2O$	0.47 mg
$Ni\ SO_4(NH_4)_2 \cdot 6\ H_2O$	0.20 mg
KI	0.09 mg
2 x distilled water	500 ml

JANSEN'S LIQUID MEDIUM	
Solution A	200 ml
Solution B	500 ml
2 x distilled water	300 ml
JANSEN'S AGAR MEDIUM	
Solution A	200 ml
Solution B	500 ml
3.33% w/v Bacto agar (Difco Co.)	300 ml

Spot Test for Virus-Sensitivity of Blue-green Algae

Four ml of a 3-day-old culture of the appropriate alga was mixed with 6 ml of melted (40°C) Jansen's agar, then poured into a 15 x 100 mm petri plate already containing 25 ml of solidified Jansen's agar. Alternatively, filamentous algae were sometime filtered onto type HA Millipore filters which were placed algae-side-up on the solidfied Jansen's agar. One drop of virus suspension, diluted to give 10^5 pfu/ml in plaque assays with S. cedrorum J3G1, was applied to a localized spot on the plate or filter. The plate was incubated as for the standard plaque assay. The host was considered virus-sensitive if lysis was observed in the spot within 14 days.

Isolation and Propagation of Cyanophage SAM-1

The virus was isolated from an untreated sewage sample entering the Oshkosh, Wisconsin, sewage treatment plant. This sewage sample would be expected to contain municipal and industrial wastes, run-off water, and a considerable fraction of chlorinated water from Lake Winnebago, since the Oshkosh municipal water comes from this lake.

The virus was isolated and cloned following a procedure modified from that of Safferman (1968). One hundred ml of the sewage sample, which had been filtered through a 0.45 µm pore-size (type HA) Millipore filter, was added to 900 ml of a 3-day-old, exponentially-growing culture of Synechococcus cedrorum J3G1 in Jansen's liquid medium. The culture was incubated at 150 rev/min, at 150 ft-c and at standard growth conditions. Lysis, characterized by bleaching and decreased turbidity of the culture, was apparent by the ninth day of incubation, at which time the culture was centrifuged at 6000 rev/min (5060 x g average) for 15 minutes, to separate cells and debris from the potentially virus-containing supernate. The supernate was filtered through a 0.45 µ Millipore filter, and stored at 4°C. The pellet was resuspending in 100 ml of 0.01 M Tris buffer, pH 8.2, containing 1% v/v of chloroform. The resuspended pellet was shaken at 200 rev/min for 10 minutes, then allowed to settle for 20 minutes at 4°C. The original supernate and the supernate from the resuspended pellet were combined, centrifuged at 5060 x g for 15 minutes, and then filtered through 0.22 µm pore-size (type GS) Millipore filter.

The filtrate was assayed for virus by the standard plaque assay with S. cedrorum J3G1 as the host organism. A viral clone was obtained by removing the agar from the center of a well-isolated plaque and suspending this virus-containing agar in 2 ml of Jansen's liquid medium. After the suspension had been stored overnight at 4°C, it was centrifuged at 5060 x g for 15 minutes and the supernate was assayed for virus by the standard plaque assay. This cloning procedure from randomly-selected, isolated plaques was repeated four times in series.

Propagation of Cyanophage SAM-1: liquid medium method--

Cultures of S. cedrorum J3G1 containing between 5×10^7 and 1×10^8 cells/ml were infected with a final concentration of 1×10^6 pfu of virus/ml, then incubated at standard conditions and 60 rev/min in the dark. After 1

hour in the dark, the culture was incubated at 100 rev/min and 150 ft-c, under standard growth conditions, for 3 to 5 days, until lysis occurred. Chloroform was added to 1% v/v. After sedimentation of the lysate at 5860 x g for 15 minutes, the supernate was filtered through a .45 μm Millipore membrane and stored at 4°C.

Propagation of Cyanophage SAM-1: plate method--

One tenth ml of a virus suspension containing 10^5 pfu/ml was added to 4 ml of a 3-day-old culture of S. cedrorum J361, then incubated in the dark at 26°C and 60 rev/min. After 1 hour, the virus-host mixture was added to 4 ml of melted (48°C) Jansen's agar, poured, and incubated as for the standard plaque assay. After confluent lysis of the host lawn developed, 10 ml of sterile 0.01M Tris buffer, pH 8.2, was dispensed onto the agar surface. The plates were incubated for 60 minutes at 26°C. The buffer and the top agar layer were carefully scraped into a 100G ml beaker. After the contents of the beaker had been allowed to settle for 75 mintues at 4°C, the buffer was carefully decanted and centrifuged at 5860 x g for 15 min at 4°C. The supernate was filtered through a .45 μm Millipore membrane and stored at 4°C.

Propagation of Cyanophages AS-1 and SM-1--

The procedures were identical to those used for cyanophage SAM-1, except that the hosts for phages AS-1 and SM-1 were S. cedrorum S1G1 and S. elongatus S2C2, respectivsly.

Stability of Cyanophage SAM-1 at Different pH Values--

Samples of Jansen's liquid medium were adjusted to different pH values by the addition of 5M NaOH or 5M HCl. Ten ml of the medium at a particular pH was added to a tube containing 0.1 ml of a virus suspension originally at pH 8. The final virus concentration was 2 x 10^6 pfu/ml. Each mixture was incubated at 26°C for 1 hour, then brought to pH 8 by a 1/10 dilution into pH 8 Jansen's liquid medium. Serial 10-fold dilutions in pH 8 Jansen's liquid medium were immediately assayed for virus by the standard plaque assay.

Multiplication Cycle of Cyanophage SAM-1: one-step growth and premature lysis experiments--

A three-day-old culture of S. cedrorum J361 in Jansen's liquid medium was diluted into an equal volume of Jansen's liquid medium supplemented with 0.1 M NaCl. This diluted culture, containing 2 x 10^8 cells/ml, was incubated at 150 rev/min, 150 ft-c and standard growth conditions. After 30 minutes of incubation, phage SAM-1 was added to a final concentration of 4 x 10^5 pfu/ml. The infected culture was incubated at 25°C and 80 rev/min in the dark, during a 1 hour "adsorption period." After the adsorption period, 1 ml samples of the infected culture were diluted 10^{-2} and 10^{-5} in Jansen's liquid medium, to prevent futher virus adsorption to host cells. The two diluted cultures, each consisting of 100 ml in a 500 ml Erlenmeyer flask, were incubated at 150 rev/min, 150 ft-c, and standard growth conditions. At regular intervals, samples were withdrawn from each culture, diluted if necessary, and assayed for plaque-forming units. Other samples from each culture were prematurely

lysed by the addition of chloroform, as follows: every 30 minutes, a 1 ml sample was removed from each culture and transferred to a 15 ml centrifuge tube containing 0.1 ml of chloroform. This chloroform-containing suspension was vigorously agitated on a Vortex-Genie mixer (Scientific Industries, Inc.) at maximum speed for 15 seconds. The suspensions were centrifuged at 6000 rpm for 5 minutes, diluted when necessary, and assayed for plaque forming units.

Sensitivity of Cyanophage SAM-1 to Chloroform and to Agitation--

A virus stock was diluted to contain 10^5 pfu/ml. Sixty ml amounts of diluted virus stock were transferred to each of nine 250 ml Erlenmeyer flasks at 4°C. Ten ml of chloroform was added to each of 3 flasks, which were then allowed to stand undisturbed for 30 min at 4°C. Ten ml of 0.01 M Tris buffer, pH 8.2, was added to each of three flasks, which were then agitated at 150 rev/min for 60 sec and subsequently allowed to stand for 30 min at 4°C. Ten ml of chloroform was added to each of three flasks, which were then agitated at 150 rev/min for 60 sec and subsequently allowed to stand for 30 min at 4°C. The infective virus in each flask was assayed using the standard plaque assay.

Partial Purification of Cyanophage SAM-1 Stocks--

Step-type sucrose gradients were prepared by carefully layering 2.0 ml of each appropriate sucrose solution (10%, 20%, 25%, 30%, 35% and 40% sucrose in 0.01 M Tris buffer, pH 8.2) into 1.6 cm x 10.2 cm cellulose nitrate centrifuge tubes (Beckman Instruments, Palo Alto, California) while the tubes were tilted 60° from the vertical. The gradients were stored undisturbed for 24 hr at 4°C, allowing diffusion to form a quasi-continuous sucrose gradient. After 24 hr, a 1.8 ml sample of a previously-cloned cyanophage stock was layered onto the top of the gradient. The tubes were placed in the SW27.1 rotor of a Beckman Model L5-50 ultra-centrifuge and centrifuged at 24,000 rpm (120,000 x g) for 20 min.

One ml fractions from the gradients were collected, scanned for absorption of ultraviolet light (at 260 and 280 nm) and analyzed for infective virus by the standard plaque assay. Fractions containing virus were pooled and dialyzed overnight against 0.01 M Tris buffer, pH 8.2. This dialyzed preparation was centrifuged a second time and dialyzed again by the same procedure. Any insoluble material was removed by centrifugation for 15 min at 1000 x g.

Electron Microscopy--

Virus suspensions which had been partially purified by sucrose-gradient ultracentrifugation and dialysis overnight against 0.01 M Tris buffer, pH 8.2, were used. A small droplet of virus suspension was deposited on the center of a carbon- and Colloidon-coated, 200 mesh copper grid. After 90 sec, and without removing any of the droplet, the grid was inverted onto a solution (at pH 4.5) of 2% w/v uranyl acetate (Mallinckrodt Chem., St. Louis, Mo.), and 0.15% w/v polymixin B sulfate (Sigma Chemicals, St. Louis, Mo.). After 5 min, all excess liquid was carefully removed by touching a piece of

filter paper to the edge of the grid. The specimen was allowed to air dry for 5 min before observation in a R.C.A. model EMU-3G transmission electron microscope.

Determination of M. aeruginosa Frequency at Different Stations and on Different Dates--

Two water samples, one from 12.7 cm below the water surface and another from 12.7 cm above the bottom, were periodically removed with a Wildo Corp. horizontal sampler, at the three stations indicated on Figure 2. These stations were: (a) station 1, at the end of Knasta's boat dock, 3.0 m from the south bank of the Fox River and 213 m upstream from the junction of the Fox River and Campbell Creek, which is Oshkosh's sewage treatment plant outlet; (b) station 2, 0.6 m from the south bank of the Fox River and 0.9 m downstream from the junction with Campbell Creek, and (c) station 3, 1.6 m from the shore of Miller's Bay in Lake Winnebago, 71 m south of the southeast corner of the Oshkosh Water Department pretreatment basin.

The M. aeruginosa cells in each sample were counted by microscopic observation in a Sedgwick-Rafter cell. If necessary, aggregates were dispersed so that individual cells could be counted. Estimates of cell concentrations obtained by this procedure are termed microscopic counts.

The M. aeruginosa cells (or aggregates) capable of forming colonies on Jansen's agar were counted as follows. Appropriate volumes of each sample, chosen to give 100-200 cyanophytes/plate, were spread on the surface of Jansen's cycloheximide agar in 15 x 100 mm petri plates. Samples containing less than 200 cyanophytes/ml were filtered onto 0.45 µm Millipore filters which were placed algae-side-up on the Jansen's cycloheximide agar. The plates were incubated at 150 ft-c and standard growth conditions. After 1 to 2 weeks, the plates were inspected at 3x or 6x magnification under a dissecting microscope and colonies typical of M. aeruginosa were counted. Counting was performed while the colonies were still small and before filamintous cyanophytes spread over the plates. Although M. aeruginosa colonies grown on Jansen's agar can be readily identified by an experienced observer, the identification's were confirmed by microscopic observation, under 1000x magnification, of cells aseptically removed from representative colonies. Each M. aeruginosa cell (or aggregate) which yielded one colony on Jansen's agar was considered to be one colony-forming unit. Estimates of M. aeruginosa concentrations obtained by this procedure are termed viable counts.

Unialgal cultures of M. aeruginosa morphological types

Several distinct M. aeruginosa colony types were observed on Jansen's-agar-containing petri plates used in obtaining M. aeruginosa viable counts. Cells from representative colonies of each colony type were aseptically removed with a flared inoculating loop and transferred into 10 ml screw-cap vials, each containing 2 ml of Jansen's liquid medium. The vials were incubated for 2 weeks, at 150 ft-c and at standard growth conditions. As soon as these cultures showed a green layer of growth at the surface of the medium, cells from the surface growth were cloned by streaking on Jansen's agar, using a flamed inoculating loop and bacteriological streaking procedures

(Rouf, 1970). The plates were inverted and incubated under standard conditions and at 150 ft-c, until colonies formed. Cells from well-isolated M. aeruginosa colonies were streaked onto another plate containing Jansen's agar medium, and this cloning procedure was continued until the culture was unialgal. In cases in which repeated streaking failed to yield unialgal cultures, a 15 ml conical centrifuge tube containing 8 ml of a culture in Jansen's liquid medium was agitated for 2 minutes at the maximum speed of a Vortex-Genie mixer, and then centrifuged at 5000 x g for 5 minutes. Most M. aeruginosa cells moved to the top of the tube, whereas other cyanophytes usually moved to the pellet. Cells were removed from the top of the centrifuge tube, resuspended in 8 ml of Jansen's liquid medium and centrifuged once more. Cells from the top of the second centrifuge tube were both streaked onto Jansen's agar and inoculated into a 10 ml screw-cap vial containing 2 ml of Jansen's liquid medium. Various combinations of centrifugation, streaking on agar and cultivation in liquid media (required periodically because M. aeruginosa does not grow indefinitely on agar media) produced unialgal cultures.

Cultures were considered unialgal if only M. aeruginosa could be observed after very careful microscopic observation of liquid cultures, including observation of capsular material. Senescent liquid cultures were carefully monitored for contaminating algae and for green growth below the surface of the medium, usually indicative of contaminating algae. The cultures were streaked on Jansen's agar, to observe for colonies not typical of M. aeruginosa.

Photography of M. aeruginosa Morphological Types--

A Pentax Co. "Spot-Matic" camera mounted on a Zeiss model RA-36 microscope and containing Ektachrome High Speed (EHI36) film was used. Specimens were illuminated from below by the microscope's light system. Colonies on agar were also illuminated from the side, by two 500 W Sylvania Photo-Ect lamps, at 30 cm from opposite sides of the specimen.

The photographed colonies on Jansen's agar had been cultured for 10 days, at 80 ft-c and at standard growth conditions. The petri plate lid was removed, a characteristic colony was centered under the microscope's low power (10x) objective and photographed. The colonies were magnified 10 times by the microscope and approximately 5-8 times by enlargement of the prints.

The cells photographed in Jansen's liquid medium had been cultured for 10 days at 150 ft-c, in 10 ml vials and at standard growth conditions. Two drops of each culture were placed on a microscope slide, covered with a coverslip, and photographed under the oil-immersion objective lens. Cells on the photographic prints were magnified 100 times by the microscope and approximately 6-8 times by enlargement of the prints.

Measurements of Dissolved Oxygen, Water Temperature and Water Clarity--

The dissolved oxygen in each water sample was determined as described by the American Public Health Association (1971). Water temperature was measured with a Yellow Springs Inst. Co. tele-thermometer, by lowering the instrument's

probe to the position of each water sample. Water trubidity was measured by raising a Secchi disc (Sloey, 1970) through the water and recording the distance at which the disc first became visible.

SECTION 3

RESULTS

The SAM-1 virus was isolated from an unchlorinated sewage sample. It was initially detected through the lysis of a laboratory culture of Synechococcus cedrorum J3, on which host it was also cloned four times in series, each time by preparing a viral stock from a well-isolated plaque.

HOST RANGE OF CYANOPHAGE SAM-1

To determine the host range of cyanophage SAM-1, plaque assays and "spot tests" involving both diluted and undiluted SAM-1 stocks were performed with a variety of algal hosts from the UW-O algal culture collection (table 3). The results are summarized in table 5. Cyanophage SAM-1 produced plaques only with Synechococcus cedrorum (strains SIGI and J3GI), Anacystis nidulans (strains AcICI and J4GI) and Microcystis aeruginosa (strain J1 only). It did not produce observable plaques with any of the other organisms tested, even when undiluted virus suspensions were used. Although phage SAM-1 was capable of lysing strain J1 of M. aeruginosa, it did not lyse M. aeruginosa strains M2 and MA.

For purposes of comparison, plaque assays and spot tests on the same hosts were also performed with cyanophages AS-1 and SM-1 cyanophages which also infect members of Chroococcales. These data are shown in table 5, which also list previously-published host range data for another cyanophage of Chroococcales, cyanophage S-1. It can be seen that the host range of cyanophage SAM-1 is related to, but distinct from, the host ranges of cyanophages AS-1, SM-1 and S-1.

The results in table 5 apply to unialgal, but not necessarily axenic cultures. The names of the nonaxenic host cultures are followed by an asterik.

PLAQUE MORPHOLOGIES AND RATES OF PLAQUE APPEARANCE ON DIFFERENT HOSTS

Cyanophage SAM-1 formed plaques with both axenic and nonaxenic cultures of S. cedrorum and A. nidulans (table 6). Axenic cultures of M. aeruginosa J1 have not been isolated. Plaques consistently became visible earlier on axenic cultures than on nonaxenic cultures of the same strain. Plaques on nonaxenic cultures exhibited bacterial microcolonies inside the otherwise clear plaques. With axenic cultures, no microcolonies were seen inside the clear plaques.

Table 6 lists the plaque morphologies produced by cyanophage SAM-1 on different susceptible hosts. For most hosts, clear plaques with sharply

TABLE 5. HOST RANGES OF SEVERAL CYANOPHAGES

Host Test Organisms	Virus			
Cyanophyta	S-1*	SM-1@	AS-1@	SAM-1#
Synechococcus strain NRC-1	+	-	-	U.R.&
Synechococcus elongatus IU 603	-	+	-	-
Microcystis aeruginosa NRC-1	-	+	-	-
Anacystis sp. IU 1548 ¢	-¢	-¢	-¢	U.R.
Anacystis nidulans IU 625, 1550	-	-	+	+
Synechococcus cedrorum IU 1191	-	-	+	+
Synechococcus sp. J-3	U.R.	-	+	+
Microcystis aeruginosa J-1	U.R.	-	-	+
Microcystis aeruginosa 1036	-	-	-	-
Microcystis aeruginosa 7005	-	-	-	-
Microcystis aeruginosa EPA	U.R.	-	-	-
Anabaena flos-aquae	U.R.	-	-	-
Nostoc muscorum	U.R.	-	-	-
Phormidium ambiguum	U.R.	-	-	-
Bacteria	S-1*	SM-1@	AS-1@	SAM-1#
Escherichia sp.	U.R.	-	-	-
Bacillus sp.	U.R.	-	-	-
Aerobacter sp.	U.R.	-	-	-
Aeromonas sp.	U.R.	-	-	-
Acaligenes sp.	U.R.	-	-	-
Proteus sp.	U.R.	-	-	-
Pseudomonas sp.	U.R.	-	-	-
Serratia sp.	U.R.	-	-	-
Streptococcus sp.	U.R.	-	-	-
Contaminants #1-#9 from Microcystis aeruginosa J1	U.R.	-	-	-

*Host range data from Safferman and Hasselton, 1973.
@Viruses kindly provided by R. S. Safferman, E.P.A., Cincinnati, Ohio.
#Isolated from sewage treatment plant, Oshkosh, Wisconsin.
¢Host range data from Safferman et. al., 1972.
&Unknown reaction (Results not available)
+Plaque formation
-No plaque formation

[redacted]

[redacted]

[redacted]

defined edges were observed. The one exception was M. aeruginosa J1, with which the plaques were turbid. Unlysed cells of M. aeruginosa, as well as bacterial cells, could be seen inside these turbid plaques.

The rate of plaque formation varied among host genera (Table 6). Cultures of S. cedrorum and of A. nidulans formed plaques in a maximum of five days under the standard plaque assay conditions. For M. aeruginosa J1, the illumination during incubation had to be decreased to 80 Ft-c. At higher illumination, the cells would yellow and die before plaques developed. At decreased illumination, turbid plaques on M. aeruginosa J1 began to develop after 12 to 15 days.

STABILITY OF CYANOPHAGE SAM-1

Clarified stocks of cyanophage SAM-1 in Jansen's liquid medium were stored at 4°C and assayed periodically by the standard plaque method. Only a slight loss in viral titer was detected after storage for 15 months.

Suspensions of phage SAM-1 showed no significant decrease in pfu/ml when stored for 24 hours at 4°C in either distilled water or 0.01 M Tris buffer, pH 8.2 (Table 7). The concentration of plaque forming units was neither increased nor decreased when phage SAM-1 suspensions were stored and absorbed to host cells in Jansen's medium supplemented with 0.1 M NaCl plus 0.001 M $MgCl_2$ (Table 7).

TABLE 7 STABILITY OF SAM-1 CYANOPHAGE IN VARIOUS SOLUTIONS

Experiment Number	Original Infectivity (pfu/ml)	Infectivity (pfu/ml) after exposure to:		
		0.1 M NaCl + 0.001 M $MgCl_2$	Distilled water	0.01 M Tris
1*	4.0×10^7	2.0×10^7	3.4×10^7	4.2×10^7
2**	7.4×10^9	4.0×10^9	7.3×10^9	8.3×10^9

* Purified SAM-1 cyanophage was diluted 100-fold with the appropriate solution; the mixtures were incubated for 24 hours at 4°C and assayed for infectivity.

** Purified SAM-1 cyanophage was dialyzed against three 1000-fold volumes of the appropriate solution, during 48 hours, and then assayed for infectivity.

Portions of a SAM-1 stock in Jansen's medium were maintained at different temperatures for 1 hour. No change in infectivity, measured by the standard plaque assay, was observed for samples held at temperatures equal to or below 45°C (Figure 3). Samples held at 50°C, 55°C and 60°C for 1 hour retained,

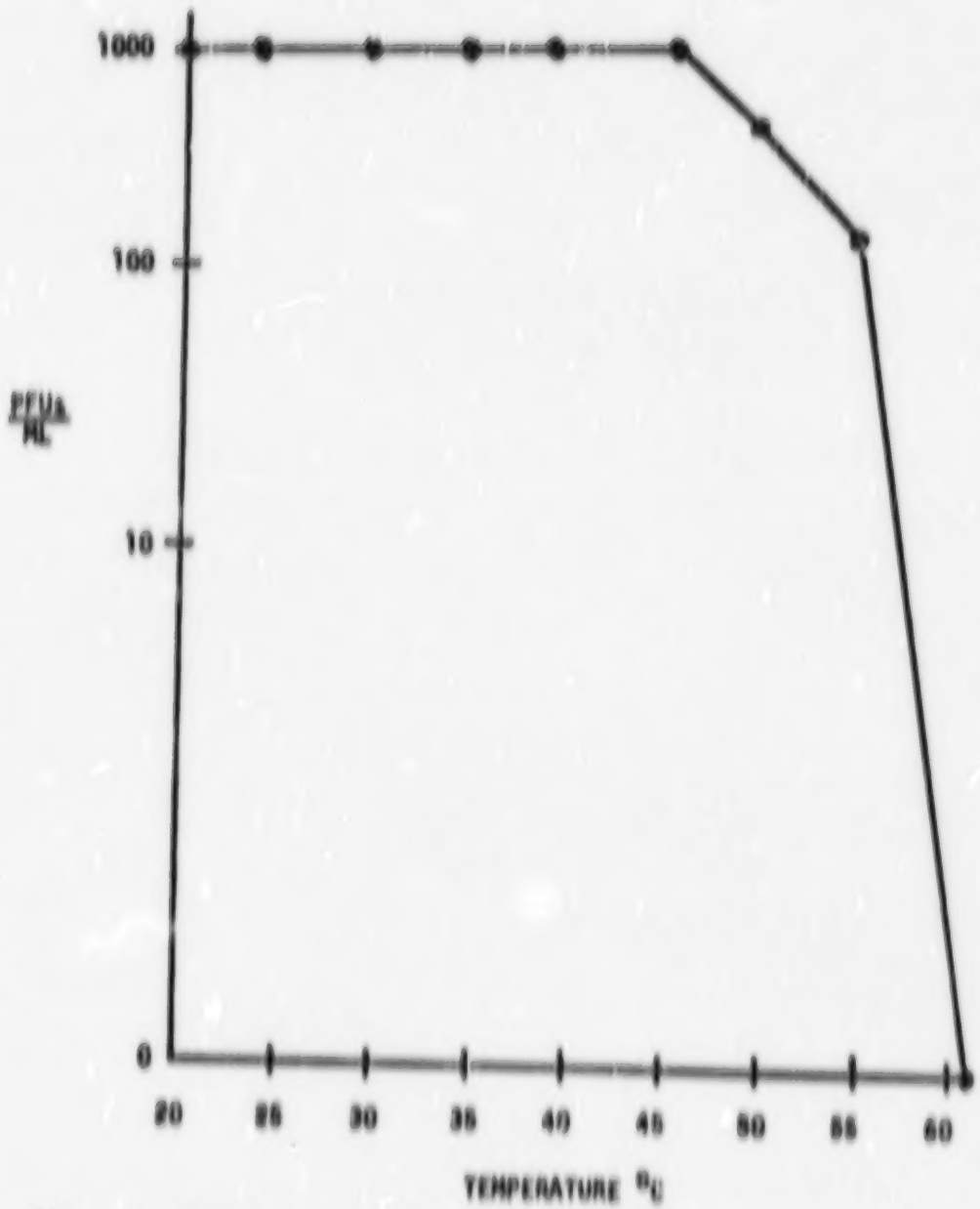

Figure 3. Stability of Cyanophage SAM-1 at different temperatures. Samples of a viral stock were maintained at appropriate temperatures for 1 hr, then assayed by the standard plate assay.

respectively, 80%, 12% and 0.0002% of the original infectivity.

Portions of a viral stock were adjusted to different pH values and stored for 1 hour at 25°C. Infectivity, measured as pfu/ml, was unaltered in all samples maintained at or between pH 4 and pH 10 (Figure 4). Samples maintained at pH 11 retained 25% of the original infectivity. Samples maintained at pH 3 or at pH 12 retained less than 0.001% of the original infectivity.

Figure 5 suggests certain synergistic effects of agitation and of chloroform on the stability of cyanophage SAM-1. When a viral stock was agitated at 150 rev/min for 60 seconds and then allowed to stand undisturbed for 30 minutes, the viral infectivity decreased to 70% of the original value. When chloroform was added to 17% v/v, no change in infectivity was observed after 30 minutes. When these same conditions of agitation and of chloroforming were applied simultaneously, the infectivity decreased to 20% of the original value.

SAM-1 MULTIPLICATION CYCLE: ONE-STEP GROWTH EXPERIMENT

The SAM-1 viral multiplication cycle was studied with a one-step growth experiment very similar to design to the original experiment of Ellis and Delbrück (1939). SAM-1 virus was absorbed to the host, S. cedrorus strain J361, for 1 hour in the dark in an absorption flask containing Jansen's liquid medium supplemented with 0.1 M NaCl. The culture was thereafter cultured under standard conditions and assayed for pfu/ml at one hour intervals for 24 hours (Figure 6). In this study, 90% of the virus adsorbed during the 1 hour dark adsorption period; the end of the adsorption period is considered to be the time when illumination was resumed. The first virus to be released from the infected cells appeared 10 hours after illumination was resumed, as is indicated by the increase in pfu/ml at that time. Thus, the latent period lasted 10 to 11 hours under these conditions. The rise period, or time of cell lysis, continued to the sixteenth hour after resumed illumination. An average of 90-100 plaque forming units of virus was released per infected cell.

SAM-1 MULTIPLICATION CYCLE: PREMATURE LYSIS EXPERIMENT

At 30-minute intervals after infection, certain samples of the same infected culture used for the one-step growth experiment were lysed prematurely by the addition of chloroform and assayed for virus by the standard plaque assay (Figure 6). In this premature-lysis experiment, no detectable virus was observed for the first 6 hr after resumed illumination, suggesting that the eclipse period of the SAM-1 multiplication cycle is 6 to 7 hr long. That intracellular virus accumulated within the host cells between 6 to 14 hr after resumed illumination is indicated by the presence of more plaque forming units in chloroform-treated, prematurely-lysed samples than in unchloroformed samples taken at the same times after illumination.

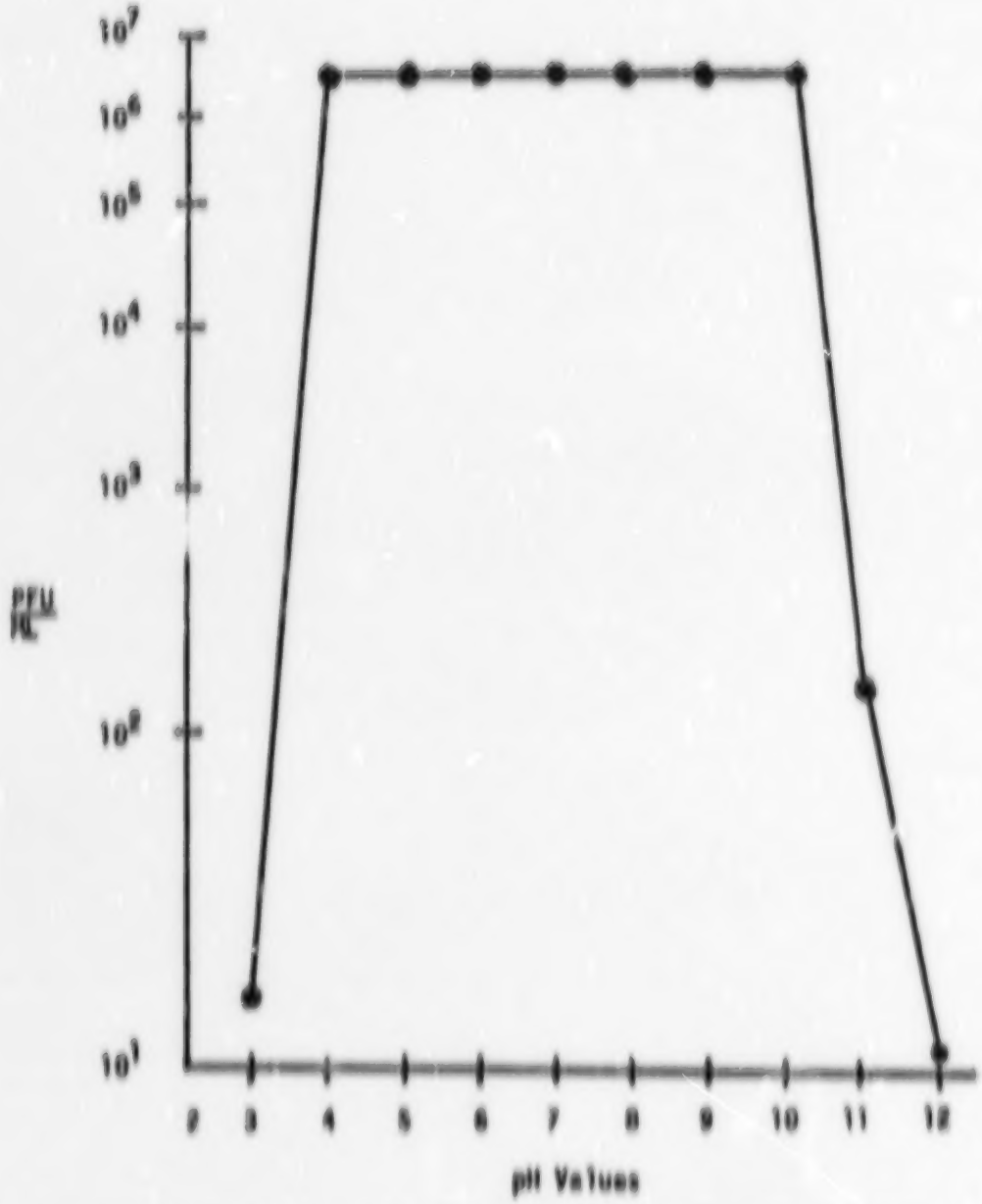

Figure 4. Stability of Cyanophage SAM-1 at different pH values. Samples of a viral stock were adjusted to the desired pH and stored at 25 C for 1 hr, then assayed by the standard plate assay.

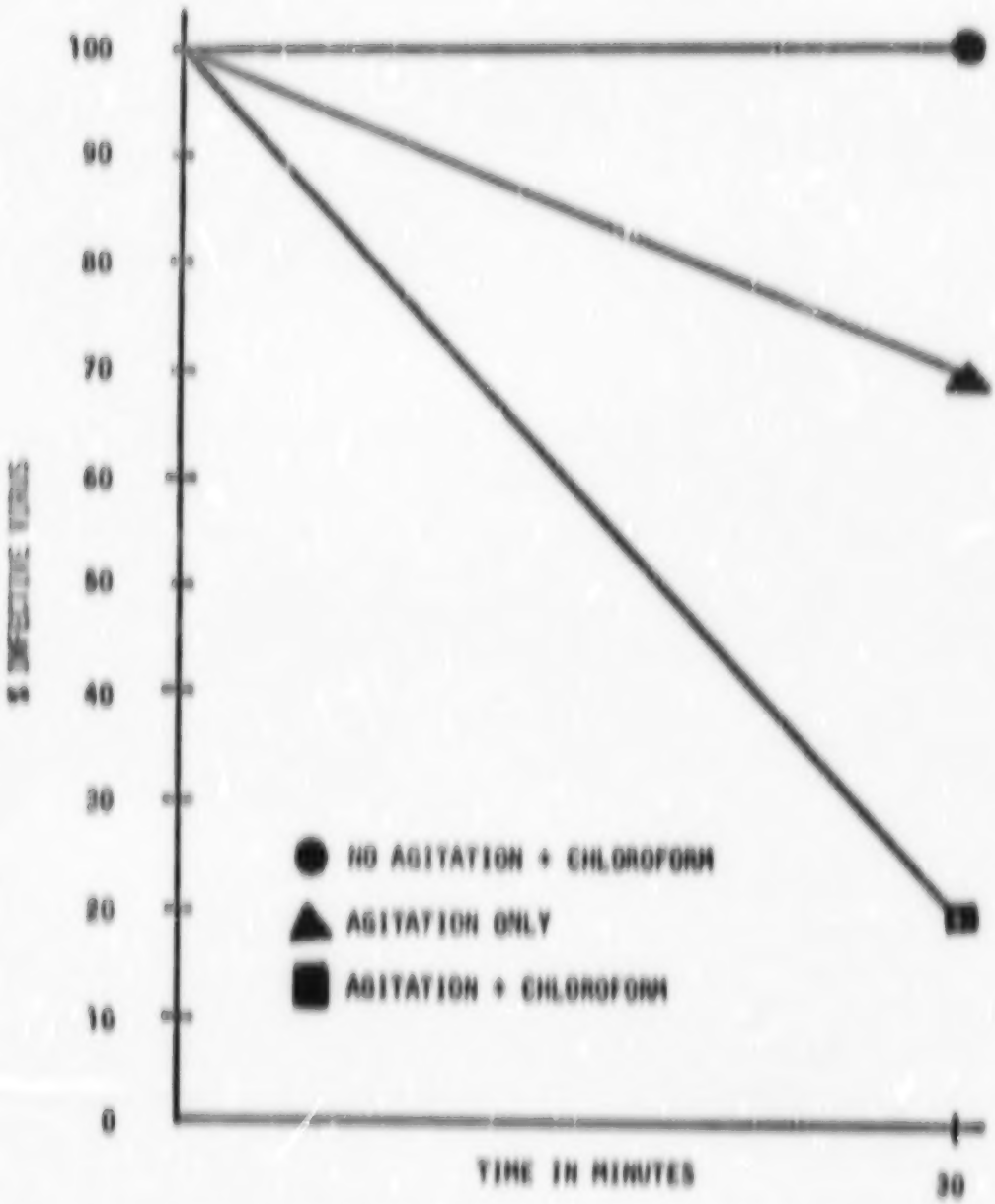

Figure 5. Sensitivity of Cyanophage SAM-1 to chloroform and to agitation. Samples of a viral stock were maintained under appropriate conditions for 30 min, then assayed by the standard plate assay.

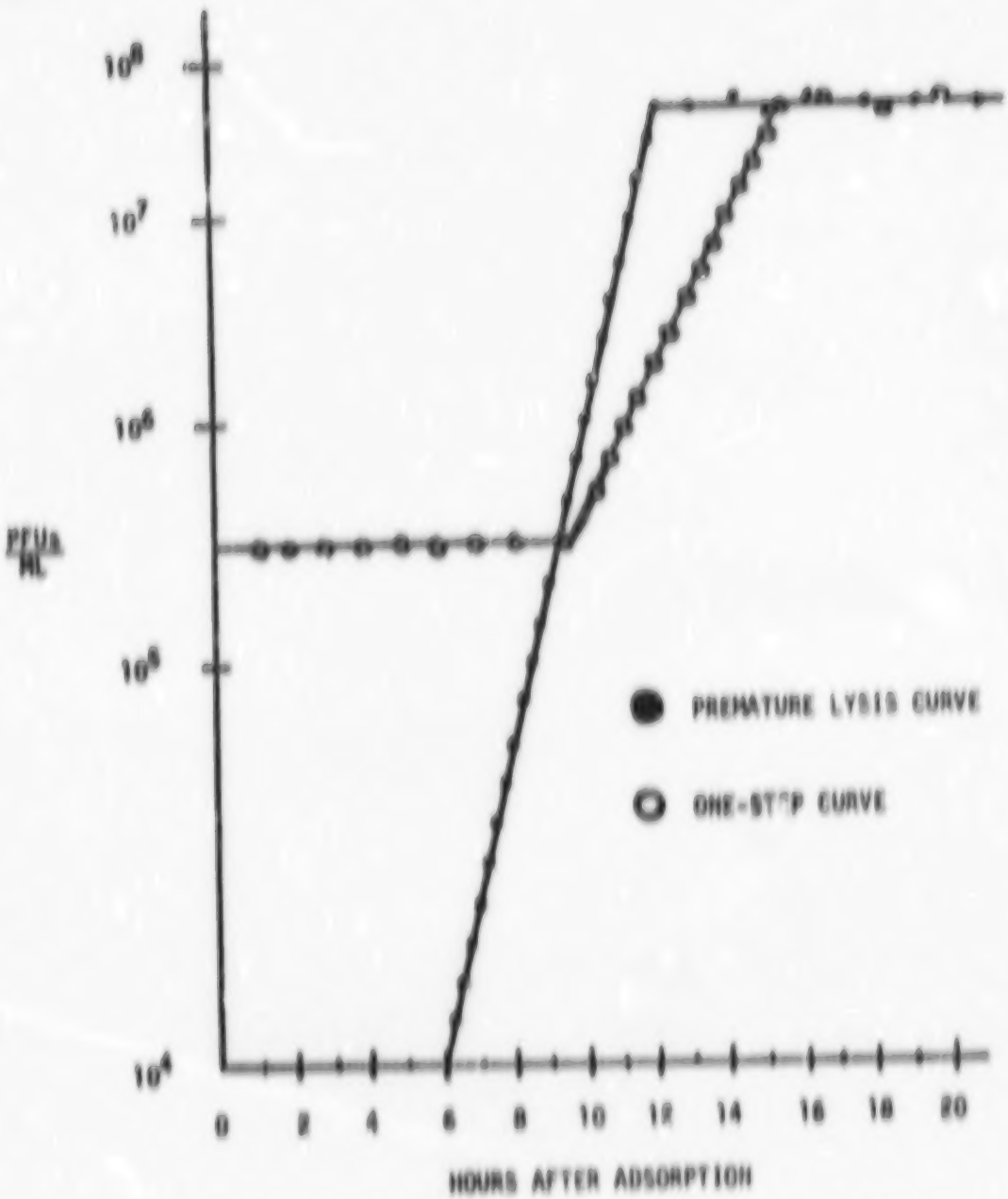

Figure 6. SAM-1 multiplication cycle, one-step growth curve and premature lysis curve, with Synechococcus cedrorum JA1 as the host.

ELECTRONMICROGRAPHS OF CYANOPHAGE SAM-1

Transmission electron micrographs of both negatively- and positively-stained viral preparations reveal phage-like particles (virions) with a polyhedral head and slightly flexible tail (Figures 7-9). The head of phage SAM-1 exhibits the familiar hexagonal outline of the other cyanophage heads. Based on various electron micrographs and preliminary measurements with calibrated polystyrene latex spheres, the head of SAM-1 virus appears to be approximately 85 nm in edge-to-edge diameter.

A long, narrow tail extends from one of the vertices of the SAM-1 head (Figures 7 and 8). The tail structure is marked by numerous stripes roughly perpendicular to the axis of the tail. In most micrographs, the tail can be observed to consist of at least three parts: an outer sheath, an inner core and a terminal structure characterized by concentric rings. Pins and fine "whiskers" may extend from the distal end of the tail. The tail sheath is frequently observed either 1/2 or 3/4 contracted (Figure 9), even though the virion is not absorbed to host cells. In such cases, the tail core can usually be observed protruding from the distal end of the tail sheath.

Although the size of the SAM-1 virion has not yet been precisely measured, ratios of the lengths and widths of various virion components can be readily determined from Figures 7 to 9 and from similar electron micrographs. Table 2 presents these ratios and compares them with comparable ratios for cyanophages AS-1, SM-1 and S-1.

PROPERTIES OF *M. aeruginosa* CULTURES FROM THE WINNEBAGO POOL

Because of the paradoxical observation (Table 5) that cyanophage SAM-1 could lyse *M. aeruginosa* strain J1, which had been isolated from the Winnebago pool, but could not lyse *M. aeruginosa* strains M2 and M4, which had been isolated from other sources, it was decided to: (a) isolate further *M. aeruginosa* cultures from the Winnebago pool and (b) determine the effect of cyanophage SAM-1 on these cultures. Several unialgal cultures of *M. aeruginosa* were isolated from the Fox River and from Lake Winnebago. Microscopic examination of these water samples suggested that more than one *M. aeruginosa* morphological type was present. Furthermore, four distinct types of *M. aeruginosa* colonies could be detected when organisms from the water samples were cultured on Jansen's agar (Table 8, Figures 10-13). Colonies of each type could readily be identified and counted, both: (a) on petri plates containing Jansen's agar medium supplemented with cycloheximide and inoculated with a fresh water sample and (b) on plates containing Jansen's agar medium and inoculated with unialgal laboratory cultures. Cells from each colony type on agar showed a characteristic cell morphology and a characteristic cell aggregation pattern when cultured in Jansen's liquid medium (Table 8, Figures 14-17). Thus, at least some of the different morphological types originally observed in the water samples appeared to retain detectable morphological differences when cultured. The existence of easily recognizable colony types on the agar medium was of particular significance, because colonies of different morphological types could be counted on plates inoculated with field samples, thus allowing quantitation of the number of colony-forming units of different *M. aeruginosa* morphological types present in a particular water sample.

Figure 7. Virion of cyanophage SAM-1.

Figure 8. Virion of cyanophage SAM-1.

Figure 9. Virion of cyanophage SAM-1, with a partially-contracted tail sheath.

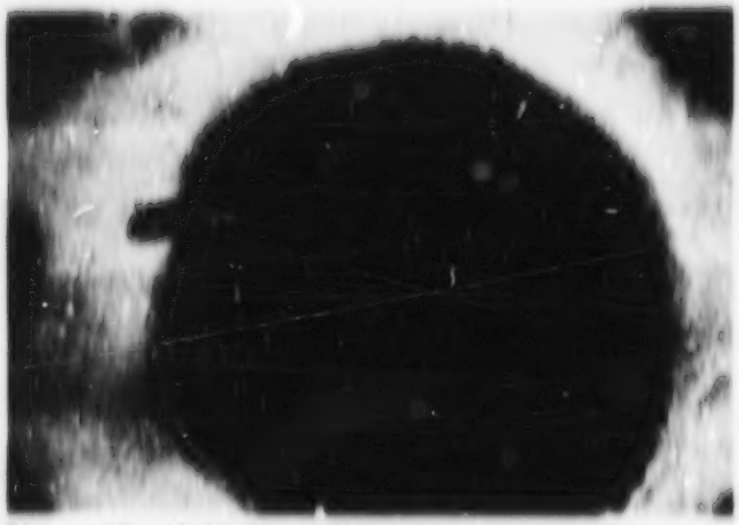

Figure 10. A 10-day colony of M. aeruginosa strain C1 (morphological type 1) growing on Jansen's agar medium. Magnified approximately 80 times, including enlargement of print.

Figure 11. A 10-day colony of M. aeruginosa strain C2 (morphological type 2) growing on Jansen's agar medium. Magnified approximately 80 times, including enlargement of print.

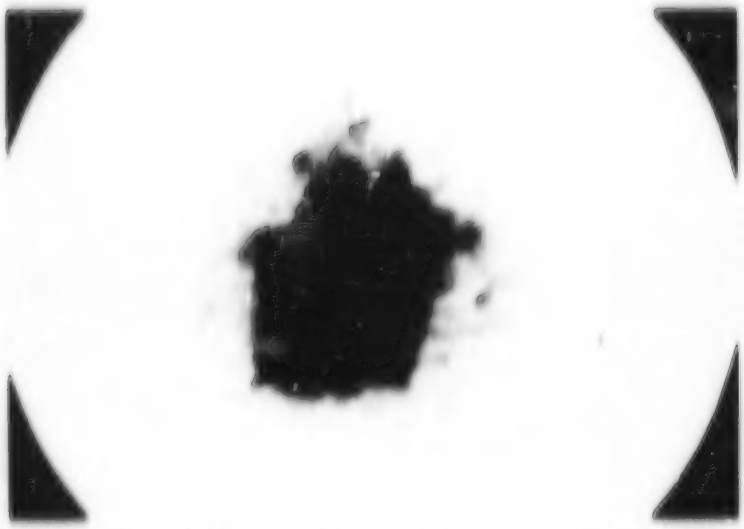

Figure 12. A 10-day colony of P. aeruginosa strain C3 (morphological type 3) growing on Jansen's agar medium. Magnified approximately 70 times, including enlargement of print.

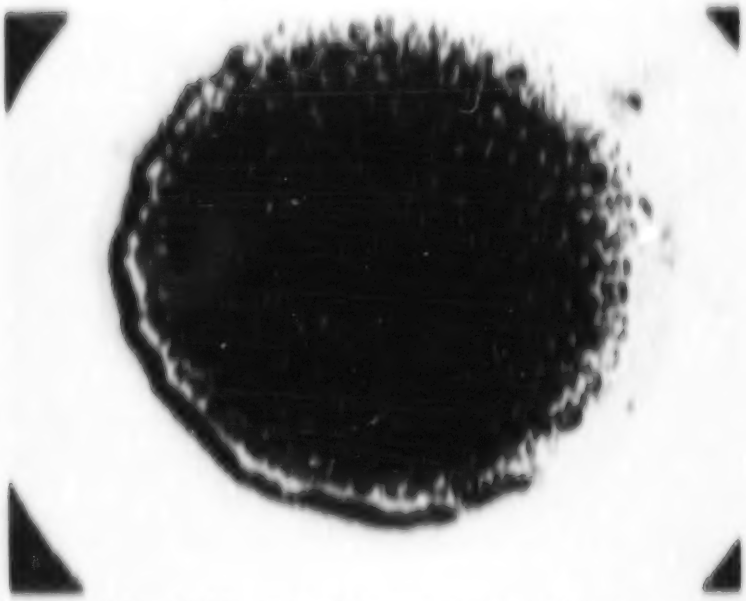

Figure 13. A 10-day colony of P. aeruginosa strain C4 (morphological type 4) growing on Jansen's agar medium. Magnified approximately 80 times, including enlargement of print.

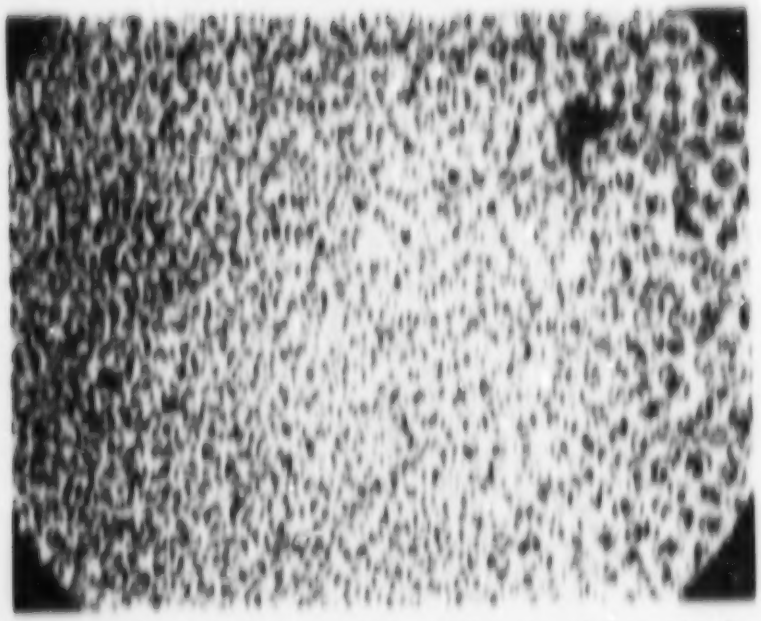

Figure 14. A 10-day culture of M. aeruginosa strain C1 (morphological type 1), growing in Jansen's liquid medium. Magnified approximately 800 times, including enlargement of print.

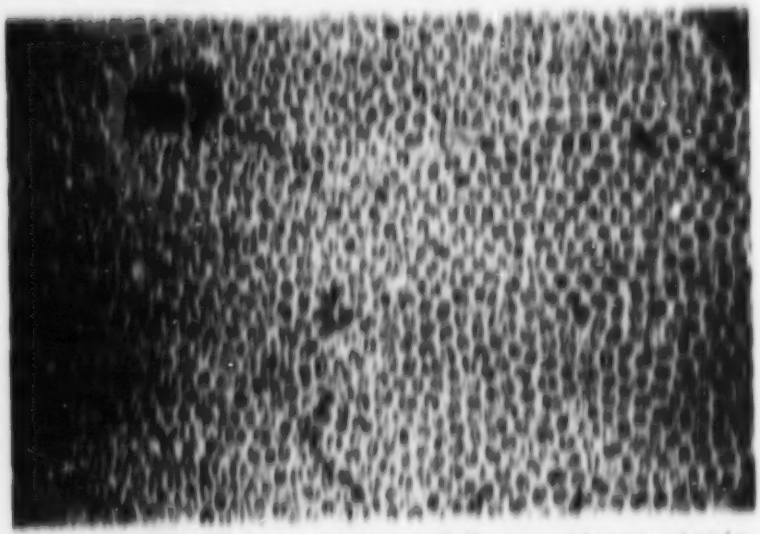

Figure 15. A 10-day culture of M. aeruginosa strain C2 (morphological type 2), growing in Jansen's liquid medium. Magnified approximately 800 times, including enlargement of print.

Figure 16. A 10-day culture of M. aeruginosa strain C3 (morphological type 3), growing in Jansen's liquid medium. Magnified approximately 600 times, including enlargement of print.

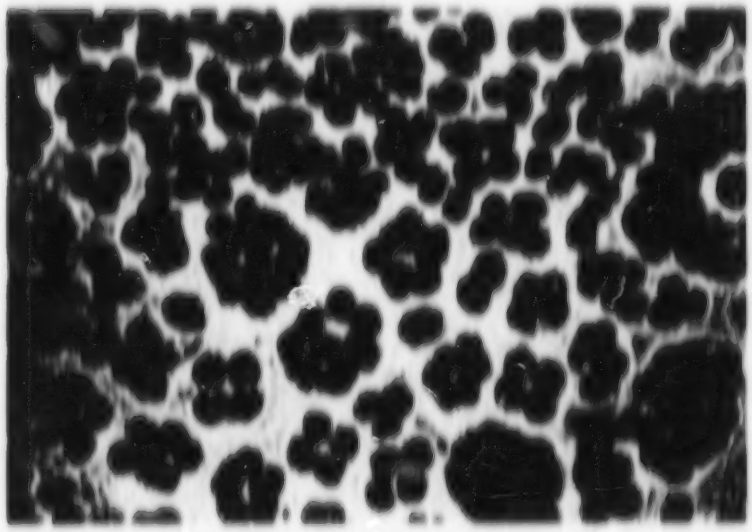

Figure 17. A 10-day culture of M. aeruginosa strain C4 (morphological type 4), growing in Jansen's liquid medium. Magnified approximately 800 times, including enlargement of print.

TABLE 8. COMPARISON OF *A. AEROGENES* MORPHOLOGICAL TYPES

		COLONY MORPHOLOGY IN JENSEN'S AGAR MEDIUM (14 day old cultures)		CELL MORPHOLOGY IN JENSEN'S LITMUS MEDIUM (7 day old cultures)		
Morphological type	Culture	Colony diameter	Mean cell diam.	Colony description	Mean cell diameter	Other Properties
1	C1	1 mm	4 μm	dark green watch not spreading	5 μm	less capsule pseudovacuoles
2	C2	2 mm	4 μm	olive green granular spreading glossy mucoid	4 μm	much capsule prominent pseudovacuoles
3	C3	3 mm	6 μm	dark green granular very glossy	7-8 μm	less capsule prominent pseudovacuoles
4	C4	4 mm	6 μm	light olive green granular very spreading glossy	5 μm	most capsule
	J1	2 mm	5 μm	olive green raised glossy	5 μm	much capsule prominent pseudovacuoles
	K2				5 μm	moderate capsule pseudovacuoles
	K4				4 μm	moderate capsule pseudovacuoles

Table 8 summarizes the colony and cell morphologies of the M. aeruginosa unialgal cultures C1, C2, C3, and C4, each of which represents one of the four observed M. aeruginosa morphological types. For comparison, Table 8 also gives the morphologies of the three M. aeruginosa strains J1, M2 and M4, strains already present in the UW-O culture collection. Figures 10 through 13 show photomicrographs of typical colonies formed on Jansen's agar by the proposed morphological types. Figures 14 through 17 present photomicrographs of 10-day-old cultures of the different morphological types grown in Jansen's liquid medium.

VIRUS-SENSITIVITIES OF M. aeruginosa MORPHOLOGICAL TYPES

Spot tests with cyanophages AS-1 and SM-1 were performed to determine the virus-sensitivities of M. aeruginosa cultures C1, C2, C3 and C4. None of these cultures showed detectable lysis when exposed to either virus.

FREQUENCY OF M. aeruginosa MORPHOLOGICAL TYPES AT DIFFERENT STATIONS AND TIMES

At four-week intervals during August through November, 1975, near-surface and near-bottom water samples were removed from two stations on the Fox River and from one station in Lake Winnebago, at the locations indicated in Figure 2. Individual M. aeruginosa cells in these samples were counted microscopically in a Sedgwick-Rafter cell, allowing calculation of the number of M. aeruginosa cells per ml of each sample (Figure 18). Although different morphological types were observed in the water samples, the morphological types were not counted separately in this preliminary study.

Appropriate volumes of each water sample were also inoculated onto Jansen's cycloheximide agar medium. The number of colonies corresponding to each M. aeruginosa morphological type were counted, allowing calculation of the number of colony-forming units (viable units) of each morphological type present in each water sample. Figures 19 through 21 give these data for stations 1 through 3 respectively.

Figure 22 gives the dissolved oxygen in each water sample. Figure 23 shows the near-surface and near-bottom water temperatures at each station and at each sampling time. Figure 24 presents water turbidity, measured as Secchi disc depth, for each station and each sampling time.

UNEXPLAINED LYTIC PHENOMENA

During the isolation of various unialgal cultures and during the maintenance of stock cultures, unexplained lysis was frequently observed. In many cases, the lytic agent formed plaques on the otherwise confluent growth of the algal host in Jansen's agar medium. Figure 25 shows the plaques produced by one such unidentified lytic agent infecting M. aeruginosa J1. Figure 26 shows areas of lysis produced by another unidentified lytic agent, occurring on the confluent growth part of a petri plate streaked with Synechococcus sp. Although these lytic agents have as yet been inadequately characterized, they are briefly mentioned here to emphasize the frequency with which lytic phenomena were observed at certain stations and to suggest possibilities for further experimentation.

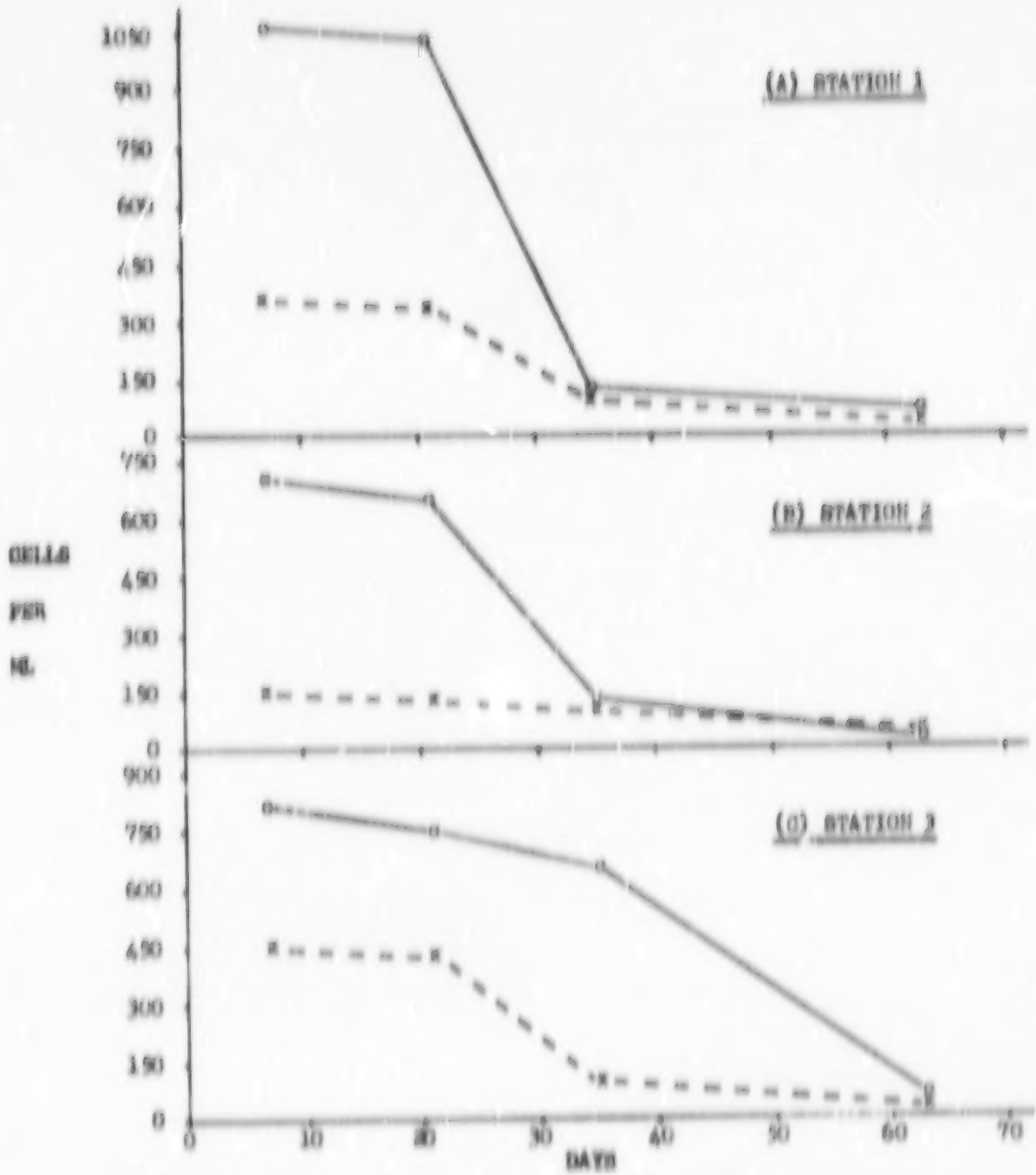

Figure 18. Frequency of M. aeruginosa cells, from direct microscopic counts. Day 0 was August 20, 1975. (A) Station 1; (B) Station 2; (C) Station 3. Symbols: ○, near-surface sample; ×, near-bottom sample. (Pilot study)

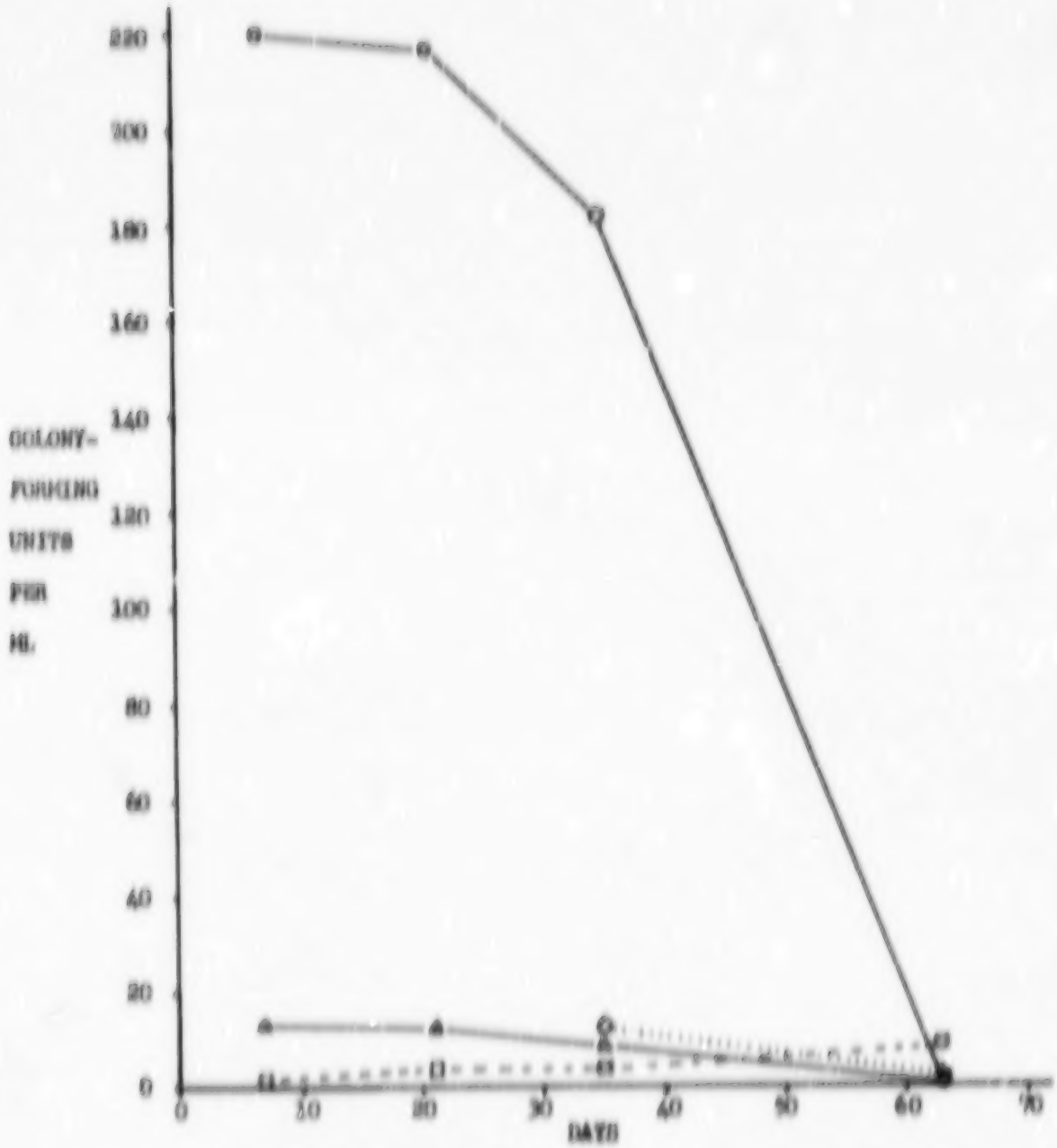

Figure 19. Frequency of *P. aeruginosa* colony-forming units, for near-surface samples removed at different times from Station 1. Day 0 was August 20, 1975. Symbols: ○, morphological type 1; □, morphological type 2; △, morphological type 3; ◇, morphological type 4. (Pilot study)

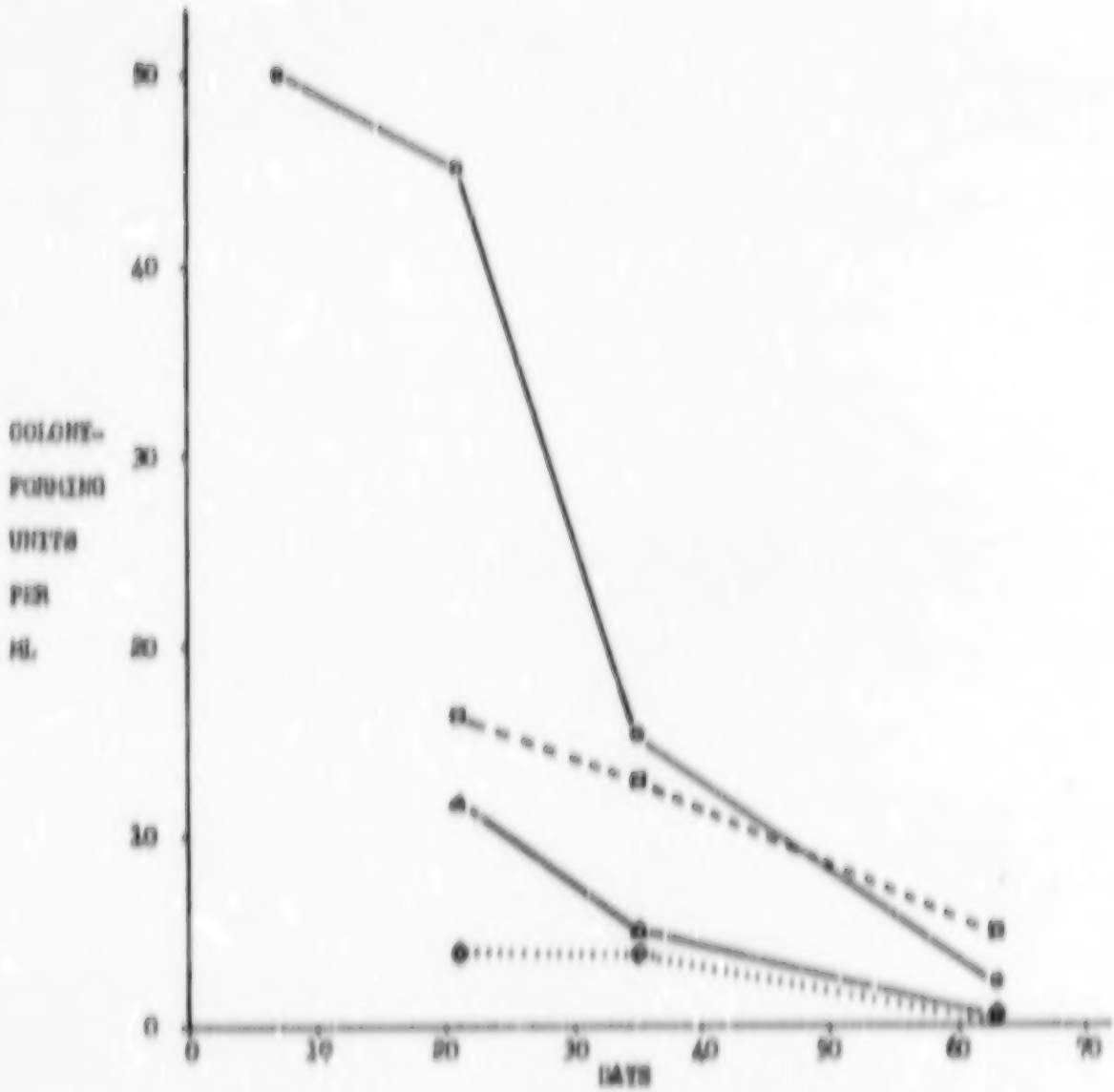

Figure 20. Frequency of P. aeruginosa colony-forming units, for near-surface samples removed at different times from Station 2. Day 0 was August 20, 1975. Symbols: ○, morphological type 1; □, morphological type 2; △, morphological type 3; ◇, morphological type 4. (Pilot study)

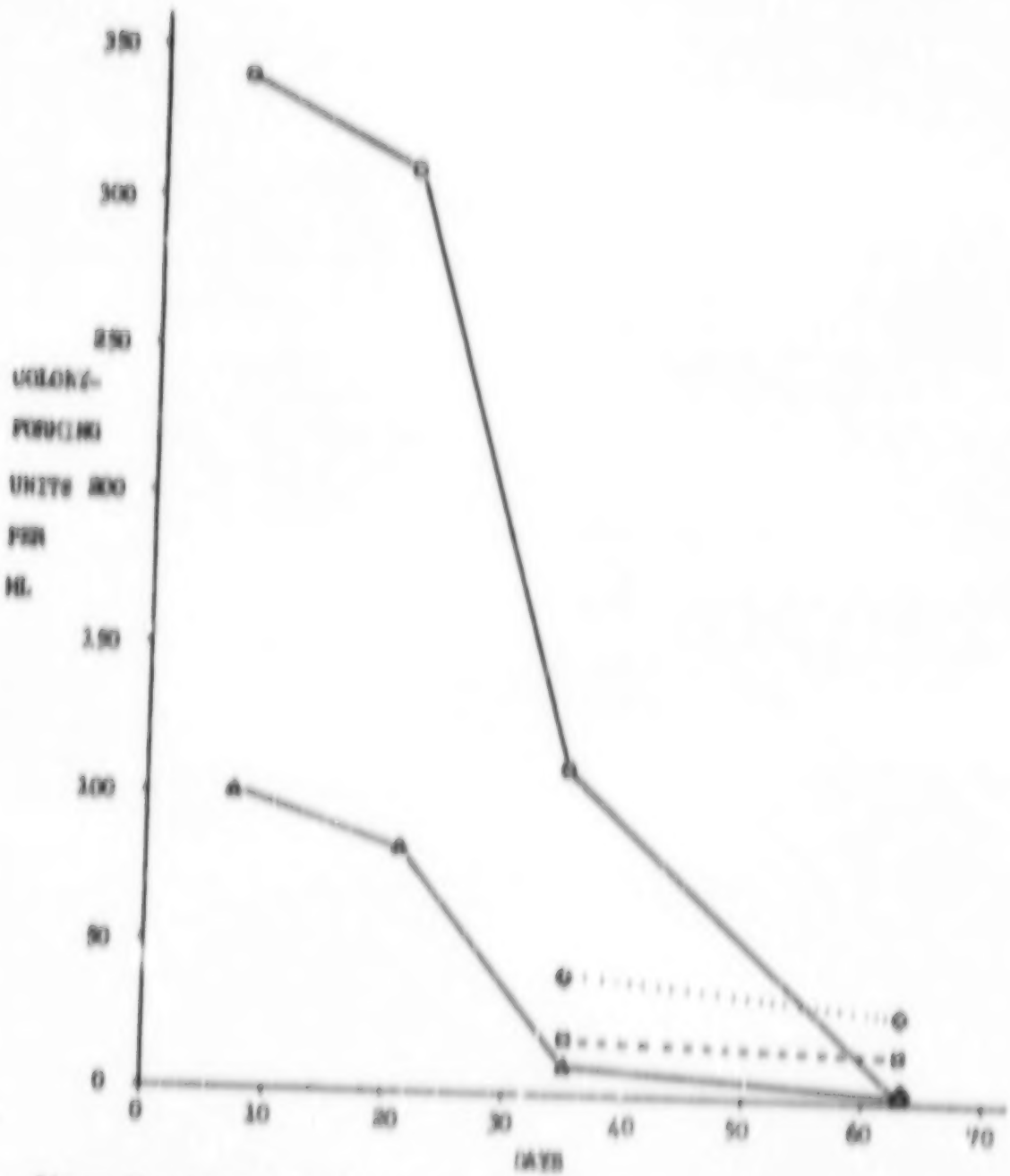

Figure 21. Frequency of P. aeruginosa colony-forming units, for near-surface samples removed at different times from Station 3. Day 0 was August 20, 1975. Symbols: ○, morphological type 1; □, morphological type 2; △, morphological type 3; ◇, morphological type 4. (Pilot study)

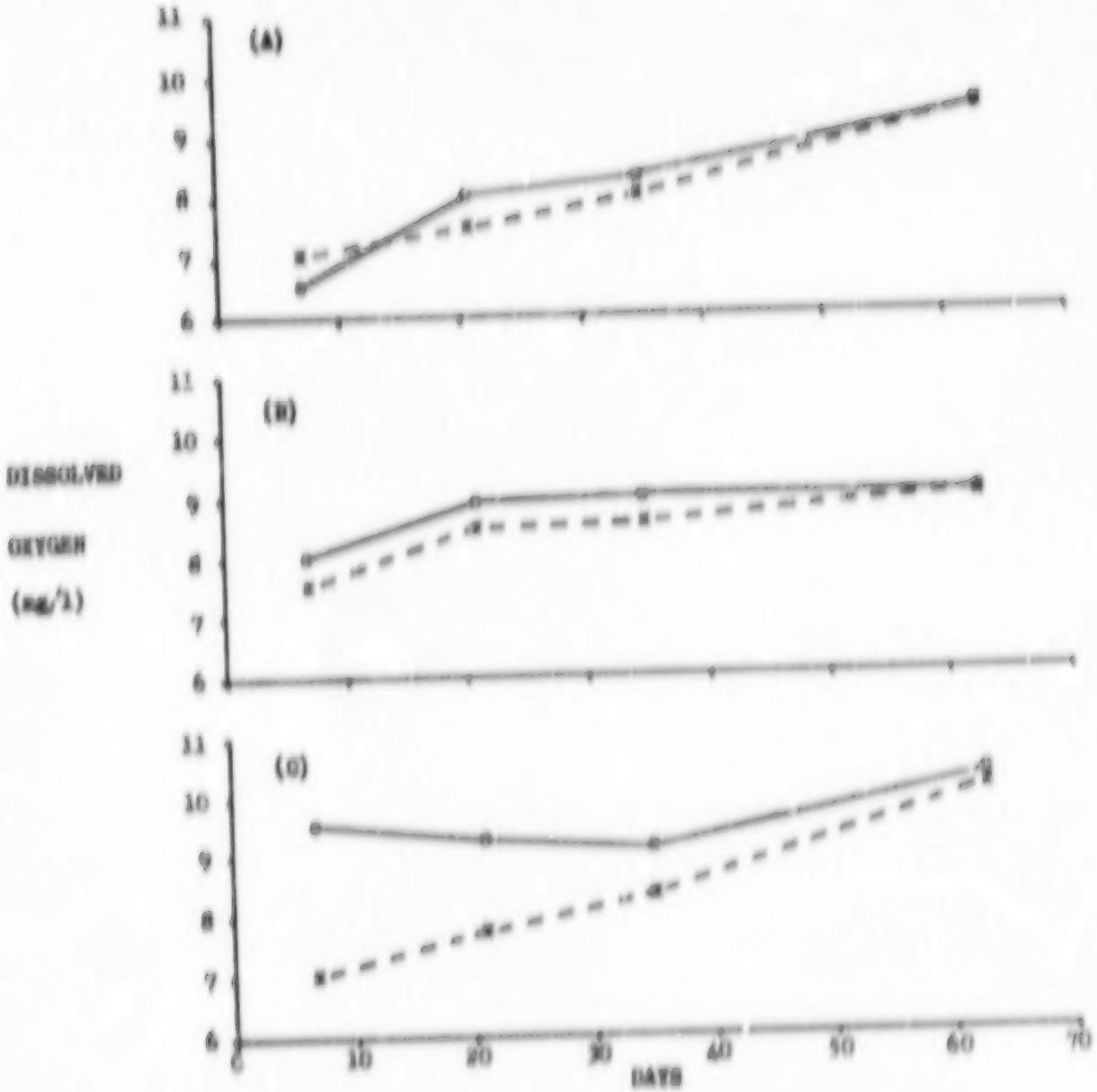

Figure 22. Dissolved oxygen in near surface and near-bottom water samples. (A) Station 1; (B) Station 2; (C) Station 3. Symbols: o, near-surface samples; x, near-bottom samples. (Pilot study) Day 0 was August 20, 1975.

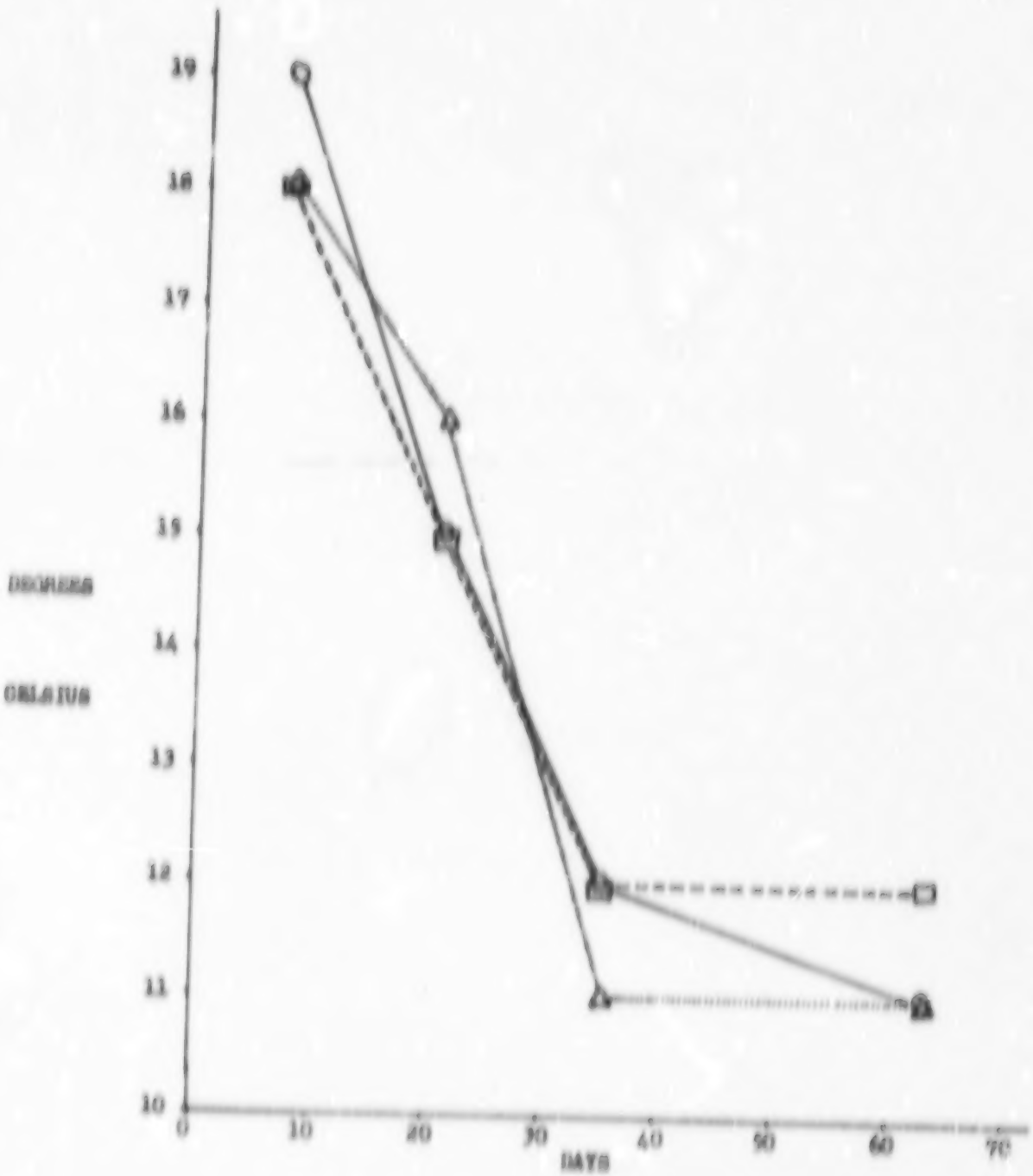

Figure 23. Water temperature at each station. Day 0 was August 20, 1975. Symbols: ○, Station 1; □, Station 2; △, Station 3. (Pilot study)

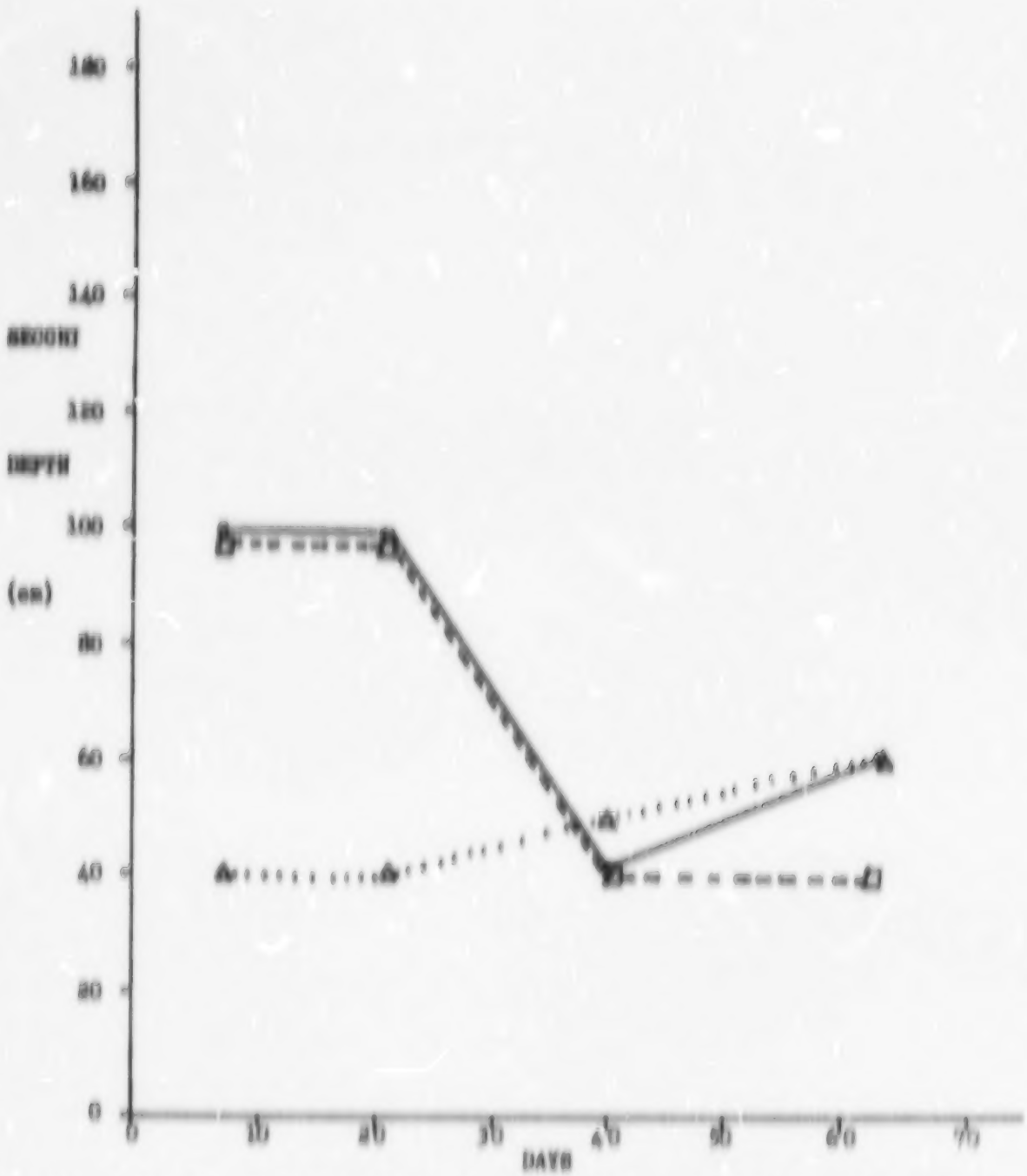

Figure 24. Secchi disc readings at each station. Day 0 was August 20, 1975. Symbols: ○, Station 1; □, Station 2; △, Station 3. (Pilot study)

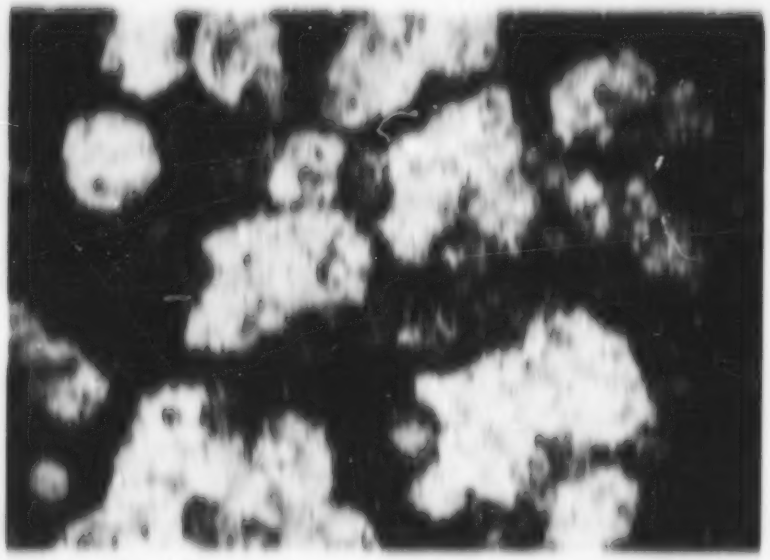

Figure 25. Plaques produced by an unidentified lytic agent infecting M. aeruginosa J1.

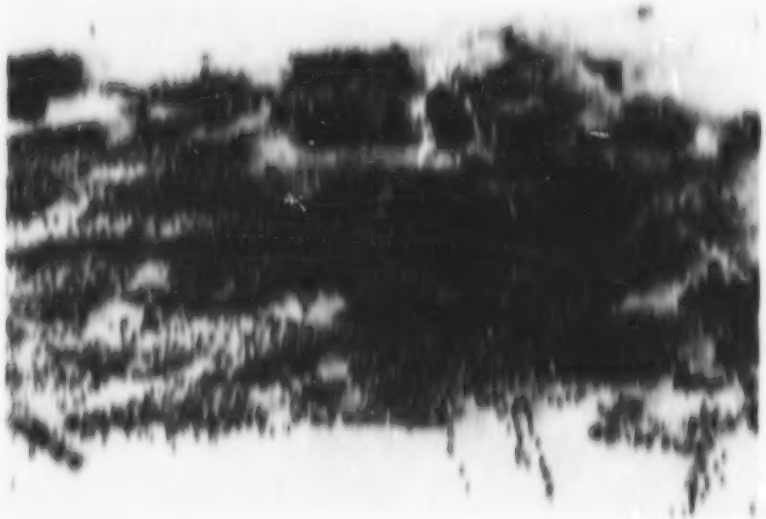

Figure 26. Plaques produced by an unidencified lytic agent infecting Synechococcus sp.

SECTION 4

DISCUSSION

PROPERTIES OF CYANOPHAGE SAM-1

The isolation and characterization of a lytic agent infecting certain blue-green algae is herein described. That this lytic agent is a virus can be concluded from: (a) its morphology as observed in electron micrographs, (b) the occurrence of an eclipse period during its multiplication cycle, (c) its filterability through 0.45 μm pore-size filters and, with lower efficiency, through 0.22 μm pore-size filters, and (d) its sedimentation properties during ultracentrifugation. Cyanophages are designated by the initials of the generic names of their hosts, to which arabic numerals are added to indicate different isolates or different subgroups (Safferman et al., 1963). Accordingly, the newly isolated virus has been designated cyanophage SAM-1, since it infects Synechococcus cedrorum, Anacystis nidulans and one strain of Microcystis aeruginosa.

Cyanophage SAM-1 differs from other reported cyanophages in virion morphology (Table 2) and in host range (Table 6). It most resembles cyanophage AS-1, but can be distinguished from this virus by differences in host range, heat-sensitivity, virion size and virion tail structure.

Some confusion exists in the taxonomy of certain SAM-1 susceptible organisms. Thus, Anacystis has been classified along with Synechococcus into typological group IA by Stanier et al. (1971), who suggested that Anacystis be reclassified as Synechococcus. Krauss (1958) has also commented that Anacystis nidulans is probably more accurately a Synechococcus. The relatedness of these two organisms is also suggested by the susceptibility of both organisms to cyanophages SAM-1, AS-1 and S-1, since most bacteriophages show a host range limited to closely related organisms (Luria and Darnell, 1967).

In contrast to cyanophages AS-1 and S-1, cyanophages SAM-1 and SM-1 infect the additional host Microcystis aeruginosa, which belongs to typological group III C, not to group IA, of Stanier et al., (1971). M. aeruginosa differs markedly from S. cedrorum and A. nidulans, in both cell morphology and growth properties. Cells of M. aeruginosa are large, 3-5 μm in diameter, pseudovacuolated, coccoid and form relatively large, mucoid colonies on Jansen's agar medium (Table 6). Cells of the latter two hosts are smaller, lack pseudovacuoles, are bacillary and form pinpoint colonies on Jansen's agar medium. M. aeruginosa cultures have remained morphologically distinct from S. cedrorum or A. nidulans cultures under a variety of growth conditions and during long-term laboratory cultivation.

The ability of cyanophages SAM-1 and SM-1 to infect not only Microcystis but also Synechococcus is of considerable interest. Future experiments may indicate that Microcystis is more related to Synechococcus than would be expected from cell morphology, plane of cell division, and colony formation. Alternatively, it may be found that the two genera are relatively unrelated, but that they nevertheless share certain viral receptors or other properties which allow infection by the same virus.

Because of the dissimilarity between M. aeruginosa and the other hosts of cyanophage SAM-1, it is prudent to critically evaluate the evidence that the virus actually infects M. aeruginosa. Cyanophage SAM-1 can be propagated by serial transfers in M. aeruginosa cultures, indicating that viral multiplication, not just cell lysis, can occur. Although rigorous verification awaits axenic cultures of M. aeruginosa, no evidence suggests that cyanophage SAM-1 infects contaminating bacteria rather than the algae or that the cultures carried contaminating type IA algae. The M. aeruginosa JI culture used for the phage-sensitivity tests had been freed of all contaminating bacteria except one organism, an Arthrobacter species, pure cultures of which are insensitive to phage SAM-1. Furthermore, none of the bacteria isolated from earlier, more heavily contaminated, cultures of strain JI could be infected with cyanophage SAM-1 (Table 6). Extreme care was taken to ensure that M. aeruginosa JI cultures did not contain other algae, including type IA organisms, which have been reported to sometimes occur as endophytes in the Microcystis envelope (Prescott, 1962). Healthy and senescent cultures were extensively examined microscopically in a search for contaminating type IA cyanophytes, either free-floating or in the mucilage surrounding M. aeruginosa cells. Cultures were streaked on agar media, to detect colonies not typical of M. aeruginosa. Liquid cultures were screened for growth below the surface of the medium, since M. aeruginosa grows at the surface whereas type IA organisms characteristically grow throughout the medium or settle to the bottom. The lawns around SAM-1 plaques were observed for contaminating type IA organisms. On the basis of these and other tests, the culture was unialgal. The sensitivity of the tests was such that they would have detected one type IA contaminant per at least 10^7 M. aeruginosa cells, a density of potential contaminants too low to account for the virus sensitivity. Nevertheless, it would perhaps be wise to further screen the M. aeruginosa JI culture for type IA contaminants, using even more sensitive procedures, to absolutely eliminate this possibility.

In contrast to the clear plaques produced by phage SAM-1 infecting S. cedrorum or A. nidulans, turbid plaques were formed by SAM-1 virus infecting M. aeruginosa. Turbid plaques could arise from several conditions, some of which are: (a) lysogeny, in which cells carry the virus in a latent, non-infectious form and are not killed by the virus, (b) poor absorption of virus to host cells, (c) failure of virus to kill host cells, (d) inability of virus to replicate in slowly-dividing senescent cells, and (e) lysis inhibition of the type observed with T-even bacteriophages (Luria and Darnell, 1967). This topic requires further investigation, especially because of its pertinence to the potential role played by cyanophage SAM-1 in the ecology of M. aeruginosa populations.

Although cyanophage SAM-1 did infect M. aeruginosa strain J1, it did not detectably infect six other M. aeruginosa strains (Table 8). Since four of the virus-insensitive strains, as well as the one virus-sensitive strain, were all isolated from the Winnebago pool, it is unlikely that any geographical factors determines why some strains are virus-sensitive. Some possible explanations of this varying virus-sensitivity are as follows: (a) the turbid plaques produced by phage SAM-1 on M. aeruginosa J1 suggest lysogeny. Lysogenic (virus-carrying) cells are usually "immune" (not lysed) by a virus which they are already carrying. If this were the case with M. aeruginosa and phage SAM-1, then most M. aeruginosa cultures would be carriers of and therefore not lysed by cyanophage SAM-1; strain J1 would be non-lysogenic and therefore virus-sensitive, (b) M. aeruginosa cultures tend to lose most of their mucilage upon prolonged laboratory cultivation. Mucilage could interfere with absorption of virus to host cells. Strain J1 had been cultured for several years when its sensitivity to phage SAM-1 was first examined; strains C1, C2, C3, and C4 had been cultured for only a few weeks and appeared to possess more mucilage. In contradiction of this idea, strains M2 and M4 were both insensitive to phage SAM-1 and yet were established laboratory strains almost devoid of mucilage when cultivated in Jansen's medium, (c) the presence of a given virus in a particular water system may exert a strong selective pressure favoring virus-resistant algal mutants. Cyanophage SAM-1 and M. aeruginosa strains J1, C1, C2, C3 and C4 were all isolated from the Winnebago pool. Strain J1 was isolated in October, 1972, approximately 7 months before cyanophage SAM-1 was; strains C1 through C4 were isolated in August and September of 1975, 2-1/2 years after phage SAM-1. Thus, strain J1 could represent a virus-insensitive strain present before phage SAM-1 became widely distributed in the Winnebago pool; strains C1 through C4 could represent virus-resistant varities which became dominant as the virus exerted selective pressure. All three of the above hypothesis (lysogeny, mucilage or mutations) can be readily tested by standard virological experiments designed to determine why some M. aeruginosa strains are virus-sensitive and others are not.

The outcome of any cyanophyte-cyanophage interaction depends not only on inherent properties of both phage and host, but also on fluctuations in external conditions and on the appearance of viral or algal mutants. Nevertheless, the cyanophage may show promise as a biological control agent. Before the potential of cyanophage SAM-1 for biological control can be evaluated, it will be necessary to understand, among other things: (a) the factors which make some M. aeruginosa strains, but not other strains, sensitive to phage SAM-1, (b) the reasons why phage SAM-1 produces turbid plaques, an indication of incomplete cell lysis, with M. aeruginosa, (c) the conditions which favor cell-to-cell transmission of the virus in nature and (d) what effects phage SAM-1 has on endogenous strains of M. aeruginosa under the environmental conditions of a particular water system.

The enumeration of M. aeruginosa morphological types by the counting of characteristic colonies grown on Jansen's cycloheximide agar offers a new method for quantitating M. aeruginosa types in water samples. Its main advantage is that it detects morphological types which "breed true" upon cultivation; it is not affected by different cell metabolic states or growth conditions in various water samples. This method quantitates "viable" M. aeruginosa cells or aggregates (with viability being defined as the ability

to grow under specific culturing conditions) and thus is a potential tool for identifying viable cells among a mixture of viable and non-viable organisms. Thus, the ratio of "total" M. aeruginosa cells (from microscopic counts) to "viable" M. aeruginosa units (from colony counts on agar media) increased with time between August and November, 1975, as the M. aeruginosa populations declined and approached senescence (Figures 18-21).

Of the four M. aeruginosa morphological types observed (Table 8), two probably correspond to F. major (Wittr.) Elenkin and two to F. minor Elenkin (Elenkin, 1924). Other important properties of the different types, including bloom formation and toxin production, remain to be elucidated.

SUGGESTED TOPICS FOR FURTHER EXPERIMENTATION

Several topics for worthwhile future experimentation are:

(1) the possibility that cyanophage SAM-1 may lysogenize (enter a latent state within) M. aeruginosa, with possible effects on the disappearance of formation of algal blooms,

(2) the factors making certain, but not all, M. aeruginosa strains sensitive to cyanophage SAM-1,

(3) refinement of the plate counting method for enumeration and identification of different M. aeruginosa morphological types,

(4) counting of different M. aeruginosa morphological types, for water samples removed at different dates and from different stations in the Winnebago pool (Figures 18 through 24 present data from a pilot study),

(5) investigation of other properties, such as bloom formation and toxin production, of the different M. aeruginosa morphological types, and

(6) further characterization of cyanophage SAM-1, including characterization of its nucleic acid and of virion proteins, identification of host cell receptor sites, investigation of antigenic cross-reactions with other cyanophages, and examinations of the effects of environmental conditions on the host-virus interaction.

SECTION 6

REFERENCES

Adolph, K. W. and R. Haselkorn. 1971. Isolation and characterization of a virus infecting the blue-green algal Nostoc muscorum. Virology. 46:200-208.

Adolph, K. W. and R. Haselkorn. 1973. Isolation and characterization of a virus infecting a blue-green alga of the genus Synechococcus. Virology. 54:230-236.

American Public Health Association. 1971. Standard Methods for the Examination of Water and Waste Water (13th ed.), Washington, D. C. 874 pp.

Birge, E. A. and C. Juday. 1914. The inland lakes of Wisconsin. Wisconsin Geology and National History Survey, Madison, Wisconsin. 137 pp.

Brown, R. M., Jr. 1972. Algal viruses, Advances Virus Research. 17:243-277.

Cannon, R. and M. Shane. 1972. The effect of antibiotic stress on protein synthesis in the establishment of lysogeny of Plectonema boryanum. Virology. 49:130-133.

Cannon, R., M. Shane and V. Bush. 1971. Lysogeny of a blue-green alga Plectonema boryanum. Virology. 46:149-153.

Cannon, R., M. Shane and E. DeMichele. 1974. Ecology of blue-green algal viruses. Jour. Environ. Eng. Div., ASCE. 100:1205-1211.

Daft, M. J., J. Bugg, and W. D. P. Stewart. 1970. A virus of blue-green algae from freshwater habitats of Scotland. New Phytol. 69:1029-1038.

Elenkin, A. A. 1924. De spec. duabus gen. Microcystis Kuetz. rutula. Not. Syst. Inst. Crypt. Horti bot. Petropol. 3:12-15.

Ellis, E. L. and M. Delbrück. 1939. The growth of bacteriophage. J. Gen. Physiol. 22:365.

E.P.A. 1974. National Eutrophication Survey working paper series Report on Lake Winnebago; Winnebago, Fond Du Lac, and Calumet Counties, Wisconsin EPA region V, working paper #57, PNERL Corvallis, Oregon.

Goryushin, V. A. and S. M. Chaplinskaya. 1966. Existence of viruses of blue-green algae. Mikrobiol. Zh (Kiev) 28:94-97. (In Russian with English summary).

Goryushin, V. A. and S. M. Chaplinskaya. 1968. Finding of the viruses of blue-green algae. "Blooming of Water", Scientific Thought Publishing House, Kiev pp. 171-174.

Jackson, D. F. 1967. Interaction between algal populations and viruses in model pools--a possible control of algal blooms. ASCE Ann Nat. Water Resources Eng., preliminary draft. 17 pp.

Jackson, D. F. and V. Sladecek. 1970. Algal viruses-eutrophication control potential. Yale Sci. Mag. 44:16-22.

Krauss, R. W. 1958. Physiology of the fresh-water algae. Ann. Rev. Plant Physiol. 9:207-244.

Luria, S. E. and J. E. Darnell, Jr. 1967. General virology, John Wiley and Sons, Inc., New York.

Olcott, P. G. 1966. Geology and water resources of Winnebago County, Wisconsin. U.S. Geol. Surv. Water Supply Paper No. 1814, U.S. Government Printing Office, Washington, D. C.

Padan, E. and M. Shilo. 1969. Distribution of cyanophages in natural habitats. 17th Congress, Intern. Soc. Applied and Theoretical Limnology. Verh. Internat. Verin. Limnol. 17:747-751.

Padan, E. and M. Shilo. 1973. Cyanophages--viruses attacking blue-green algae. Bacteriol. Reviews 37:343-370.

Padan, E., M. Shilo and N. Kislev. 1967. Isolation of "cyanophages" from freshwater ponds and their interaction with Plectonema boryanum. Virology 32:234-246.

Padan, E., M. Shilo and A. B. Oppenheim. 1972. Lysogeny of the blue-green alga Plectonema boryanum by LPP-2 SPI cyanophage. Virology 47:525-526.

Prescott, G. W. 1970. Algae of the Western Great Lakes. Wm. C. Brown, Pub., Dubuque, IA. 978 pp.

Rimon, A. and A. B. Oppenheim. 1975. Heat induction of the blue-green alga Plectonema boryanum lysogenic for the cyanophage SPI cts 1. Virology 64:454-463.

Rouf, M. A. 1970. Laboratories exercises in general microbiology. Castle Pierce Printing Company, Oshkosh, Wisconsin. 128 pp.

Safferman, R. S. 1960. Virus diseases in blue-green algae. In Daniel F. Jackson (ed.), p. 429-439. Algae, man and the environment. Syracuse University Press, New York.

Safferman, R. S. 1973. Phycoviruses. In The Biology of Blue-green Algae. N. G. Carr and B. A. Whitton, eds., Blackwell Sci. Pub., Oxford.

Safferman, R. S., T. O. Diener, P. R. Desjardins and M. E. Morris. 1972. Isolation and characterization of AS-1, a phycovirus infecting the blue-green algae Anacystis nidulans and Synechococcus cedrorum. Virology 47:106-113.

Safferman, R. S. and M. E. Morris. 1963. Algal virus: isolation. Science 140:679-680.

Safferman, R. S. and M. E. Morris. 1964. Control of algae with viruses. J. Amer. Water Works Assoc. 56:1217-1224.

Safferman, R. S. and M. E. Morris. 1967. Observations on the occurrence, distribution and seasonal incidence of blue-green algal viruses. Appl. Microbiol. 15:1219-1222.

Safferman, R. S., M. E. Morris, L. A. Sherman and R. Haselkorn. 1969. Serological and electron microscopic characterization of a new group of blue-green algal viruses (LPP-2). Virology 29:775-780.

Safferman, R. S., I. R. Schneider, R. L. Steere, M. E. Morris and T. Diener. 1969. Phycovirus SM-1: a virus infecting unicellular blue-green algae. Virology 37:386-395.

Shano, M. S. 1971. Distribution of blue-green algal viruses in various types of natural waters. Water Research 5:711-716.

Sherman, L. A. 1975. Replication and control of AS-1. Abstr. Conf. on Blue-green Algae and their Viruses, Newark, Delaware.

Shilo, M. 1971. Biological agents which cause lysis of blue-green algae. Mitt. Internat. Verin. Limnol. 19:206-213.

Shilo, M. 1972. The ecology of cyanophages. Ramidgeh 24:76-82.

Singh, P. K. 1973. Occurrence and distribution of cyanophages in ponds, sewage and rice fields. Arch. fur Mikrobiol. 89:169-172.

Singh, R. N. and P. K. Singh. 1967. Isolation of cyanophages from India. Nature 216:1020-1021.

Singh, R. N. and P. K. Singh. 1972. Transduction and lysogeny in blue-green algae. In Taxonomy and Biology of Blue-green Algae, T. V. Desikachary, Ed., Univ. of Madras Press, pp. 250-261.

Singh, R. N., P. K. Singh and P. K. Varanasi. 1969. Lysogeny and induction of lysis in blue-green algae and their viruses. Proc. 56th Int. Sci. Congress, p. 272.

Stoxy, W. E. 1970. The limnology of hypereutrophic Lake Butte des Morts, Wisconsin Proc. 13th Conf. Great Lakes Research, 951-960.

Stoey, W. E. and J. Blum. 1972. The algae of the Winnebago Pool and some tributary waters. Trans. Wisconsin Acad. Sci. Arts Letters. 60:125-145.

Stanier, R. Y., R. Kunisawa, M. Mandel and G. Cohen-Bazire. 1971. Purification and properties of unicellular blue-green algae (order Chroococcales). Bacteriol. Rev. 35:171-205.

Starr, R. C. 1964. The culture collection of algae at Indiana University. Amer. J. Bot. 51:1013-1044.

Williams, R. E. O. and J. L. Rippon. 1953. Bacteriophage typing of strains of Staphylococcus aureus from various sources. Lancet 264:510-514.